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STATEMENT OF INTEGRITY

I hereby declare having conducted my thesis with integrity. I confirm that I have not used plagiarism or any form of falsification of results in the process of the thesis elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

A homeostase endócrina e o envelhecimento saudável – um estudo comparativo baseado em distintos padrões populacionais de cognição

Resumo

O aumento da longevidade humana durante o século passado transformou o estudo do envelhecimento num dos tópicos mais relevantes da atualidade. A manutenção das capacidades cognitivas ao longo da vida é de extrema importância para os próprios indivíduos e para toda a sociedade. A homeostase endócrina é fundamental na coordenação das respostas adaptativas de muitos sistemas biológicos, incluindo do cérebro. Neste estudo avaliámos longitudinalmente vários eixos endócrinos [com ênfase na vitamina D e nos eixos hipotálamo-hipófise-supra-renal, hipotálamo-hipófise-tiroide e somatotrópico (*growth hormone/insulin-like growth factor I* (GH/IGF-1)] em indivíduos saudáveis com mais de 55 anos de idade e a sua relação com o desempenho cognitivo. Os dados obtidos indicaram não haver ligação entre 25(OH)-vitamina D, cortisol matinal e noturno, TSH e T_4 livre e o desempenho funcional e de memória, tanto na análise transversal como na longitudinal. Identificámos que o declínio normal do IGF-1 durante o envelhecimento se associa a um pior funcionamento executivo. Paradoxalmente, nalguns indivíduos, os níveis estáveis baixos de IGF-1 estavam transversalmente associados a um melhor funcionamento executivo, e os níveis mais elevados de GH pós-exercício estavam longitudinalmente associados a pior desempenho nestas tarefas. A relação entre o sistema endócrino e o desempenho neuro-cognitivo durante o envelhecimento está baseada numa complexa teia de interações. Indivíduos saudáveis ao envelhecerem podem apresentar capacidades cognitivas e "assinaturas" endócrinas que estão em parte cristalizadas e que provavelmente representam uma de várias características biográficas únicas. Torna-se, assim, difícil de determinar (em ambientes estáveis e não-patológicos) qualquer influência mais significativa entre uma normal variação hormonal e o funcionamento do cérebro. Os resultados aqui apresentados parecem concordar com essa premissa. São necessários mais estudos para esclarecer quais as características endócrinas "normais" que são verdadeiramente mal-adaptativas ao longo do tempo. Para tal tarefa será necessário incluir coortes de acompanhamento mais longas com avaliações endócrinas e cognitivas frequentes e precoces, a fim de determinar padrões normais do trilho individual no caminho do envelhecimento saudável.

Palavras-chave: 25-(OH)D, Cognição, Cortisol, Envelhecimento, GH, IGF-1, T_4 livre, TSH.

Endocrine homeostasis and healthy ageing - a comparative study based on distinctive cognitive population patterns

Abstract

The rise of human longevity during the last century turned ageing a presently major topic in research. Maintenance of cognitive abilities during this life period is of paramount importance for individuals and society itself. Endocrine homeostasis plays a fundamental role in adaptative responses of most biological systems, including the brain. The aim of this project was to assess various endocrine axes [with emphasis in their vitamin D status, hypothalamus-pituitary-adrenal, hypothalamus-pituitary-thyroid and growth hormone/insulin-like growth factor I (GH/IGF-1) somatotropic axes] in healthy individuals older than 55-years of age, and their relationship with cognition. This work recognized no overall association between 25(OH)-vitamin D, morning and nocturnal-cortisol, TSH and free T_4 levels with cognitive executive and memory composite scores, both in cross-sectional and longitudinal analysis. We observed that normal decline of IGF-1 during ageing was associated to worse executive functioning. Paradoxically we also found that some individuals with stable low IGF-1 levels were cross-sectionally associated to better executive functioning and that post-exercise GH levels were negatively related to longitudinal executive performance.

Endocrine crosstalk with neurocognitive performance during ageing is based on a multifaceted structure. Healthy individuals during this life period may present many cognitive skills and endocrine signatures that are in part crystalized, which probably represent their biographical portrait. Therefore, in steady-state settings it may be difficult to determine any significant influence between normal hormonal variation and brain functioning in healthy cognitive individuals. The results presented are in agreement with this assumption. Further studies are needed to clarify which early "normal" endocrine features are truly mal-adaptative over time. These will have to include longer follow-up cohorts with regular and earlier endocrine and cognitive assessments, in order to determine normative individual tracks during the ageing path.

Keywords: 25-(OH)D, Ageing, Cognition, Cortisol, Free T_4, GH, IGF-1, TSH.

List of abbreviations

1,25(OH) 2D	1,25-dihydroxyvitamin D (calcitriol)
25(OH)D	25-hydroxyvitamin D (calcidiol)
ACC	Anterior cingulate cortex
ACTH	Adrenocorticotropic hormone
ADP	Adenosine diphosphate
AMPK	5' adenosine monophosphate-activated protein kinase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APOE	Apolipoprotein E
ARF	Alternate reading frame
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
BCE	Before current era
CA1	Cornu ammonis 1
CAR	Cortisol awakening response
CBG	Corticosteroid binding protein
CDK	Cyclin-dependent kinases
CETP	Cholesteryl ester transfer protein
CI	Confidence interval
CNS	Central nervous system
COWAT	Controlled oral word association test
CpG	5'-Cytosine-phosphate-Guanine-3'
CRH	Corticotropin releasing hormone
CYP2R1	Vitamin D 25-hydroxylase (cytochrome P450 family 2 subfamily R member 1)
CYP27B1	25-Hydroxyvitamin D 1-α-hydroxylase (cytochrome P450 family 27 subfamily B member 1)
DCS	Diurnal cortisol slope
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulphate

DNA	Deoxyribonucleic acid
DSST	Digit symbol substitution test
e.g.	Exempli gratia (for example)
EHLEIS	European health and life expectancy information system
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinases
EU	European Union
fMRI	Functional magnetic resonance imaging
FOXO	Forkhead box O
GFR	Glomerular filtration rate
GH	Growth hormone (somatotropin)
GR	Glucocorticoid receptor
GREs	Glucocorticoid response elements
HPA	Hypothalamic-pituitary-adrenal
HPT	Hypothalamic-pituitary-thyroid
IGF-1	Insulin growth factor 1
IGF-1R	Insulin growth factor 1 receptor
INK	Inhibitors of CDK
i.e.	id est (in other words)
IL	Interleukin
MAPK	Mitogen-activated protein kinases
MCI	Mild cognitive impairment
miRNA	Micro ribonucleic acid
MMSE	Mini-mental state examination
MR	Mineralocorticoid receptor
mTOR	Mammalian target of rapamycin
NAD	Nicotinamide adenine dinucleotide
NF-κB	Nuclear factor κ-light-chain-enhancer of activated B cells
OCDE	Organisation for economic co-operation and development
OR	Odds ratio

PFC	Pre-frontal cortex
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus
QALYs	Quality-adjusted life-years
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SIRT	Sirtuin gene
SMD	Standardized mean difference
SRT	Selective reminding test
TFGβ	Transforming growth factor β
UFC	Urinary free cortisol
UN	United Nations
USA	United States of America
UVB	Ultraviolet B radiation
VDBP	Vitamin D binding protein
VDR	Vitamin D receptor
WHO	World Health Organization

Contents

How can the past and future be, when the past no longer is, and the future is not yet?

As for the present, if it were always present and never moved on to become the past, it would not be time, but eternity.

Augustine of Hippo, 354-430 AD.

1. Introduction

Time, in classical terms, is one of the fundamental scalar quantity measures that flows in an apparent unidirectional way, consent an indefinite continued progress of existence and allows events in the past, present, and future to be perceived as a whole (Oxford 2011, Muller 2017).

Likely since our first beginning as a cognitive species *Time* was recognized as the root of our proper existence. In the second millennium before common era, the *Atharvaveda* (a gathering of hymns that forms part of the ancient sacred literature of the Hindus) already portraited *Time* as the creator and ruler of everything (2018).

Time and *Entropy* (from the Greek ἐντροπία - a turning towards, a transformation content - the measure of the disordering of things) are intrinsically linked. Entropy was lower in the past and it will be higher in the future – with more disorder and therefore more odds. For some, this *Entropy* flow from order to disorder is what gives mankind the perception of *Time* (Rovelli 2018).

The construct of awareness of consecutive events and temporal rhythms is dependent of several physical and biological variables. Classical experiments on this subject have led to discovery of distinct individual estimation of time between events, but without fundamentally changing the effect of *Time* on their molecular and biological systems (O'Hanlon, McGrath et al. 1974).

What are the true effects of *Time* on the biological systems?

Is it Ageing?

1.1 What is Ageing?

Definition

Ageing was perceived differently during Human history, but today it retains a negative connotation. The ancient Greek myth of *Thitonus* (lover of *Eos* – the personification of dawn) was graced by *Zeus* with eternity, but without the desired eternal youth (*id est* healthy ageing) and finished transformed in a cicada until eternity. This saga tell us much about how ageing was then perceived to be a curse, linked to the decaying effects of *Time* on the somewhat universal human wish for immortality – and of course also about how the longevity of the cicada insect was mysterious and unexpected for the Ancient Greeks (Kalogeraki 2005).

Ageing, if used as synonym of *Senescence*, is a biological autonomous process of deterioration that the living organisms seem to undergo with increasing chronological age after the initial period of development (Rose 1991, Gaillard and Bonenfant 2008). This deterioration is characterized by a progressive loss of physiological and homeostatic integrity, leading to a weakened function and amplified susceptibility to death. It is only one of the phenomena that portray the finitude of life (ageing, determinants of longevity, age-associated diseases and death) and often confused with some of them.

Longevity is now perceived as a completely different process from *Ageing*. It is measured as the time between birth and death. Biological systems have probably included this process following the evolution of sexual reproduction when, after the virtual “immortality” seen in the asexual reproduction (with the same organism being cloned indefinitely), the individual became "disposable" and thus “mortal” (Williams 1957). Its potential effect can only be determined after reaching reproductive maturity, *i.e.* outside the genome’s main purpose, and present an extraordinary diversity across distinct species and individuals. For example, in animals, lifespans can range from an estimated 1,400 years for the hydra to just 25 days for nematode worms (Jones, Scheuerlein et al. 2014). Despite the weak correlation between length of life and degree of senescence demonstrated by some authors, the phylogenetic affinity between the two seem to have had some role during the evolution of species (Jones, Scheuerlein et al. 2014). Taxonomic clustering of mortality, fertility and survivorship patterns is observed in mammals but is not so clear within invertebrates, vascular plants and others like corals, which are scattered across the continuum of senescence and may actually continue to grow even after reproductive maturity with constant, or slowly decreasing, fertility and mortality rates.

Unlike disease, that only affects a fraction of the population (even in healthy elderly), ageing affects every multicellular life that reaches a fixed point at reproductive maturity and across all species barriers, probably through the same universal molecular aetiology, whether it is programmed or stochastic (Hayflick 2007).

Currently *Senescence* should be viewed as a life-history time-dependent process that is shaped by natural selection and occurs even though it should be selected against. This evolutionary explanation embraces many non-mutually exclusive hypotheses that have been created to explain this apparent paradox.

Current theories about ageing

Today there are more than 300 different theories about ageing (Medvedev 1990). Two main categories are currently accepted, both providing explanations to the non-exclusive biological and molecular changes seen during ageing: the evolutionary (or biological) and the chemical hypotheses.

The **evolutionary** (or biological) **hypothesis** encompasses two main theories:

The *mutation accumulation theory* derives from the seminal work of Medawar in 1946 where he first stated that attenuation of natural selection seen during ageing will inevitably lead to the loss of genetic control over the later part of the life span (Medawar 1946). For this reason, a lethal germline mutation that affects only individuals after the reproductive maturity will not experience selection and will have already passed it to their offspring by the age of the pathologic expression. Over generations, these late-acting deleterious mutations will tend to accumulate and potentiate the decline of homeostatic systems and therefore will increase mortality rates during ageing.

The *antagonistic pleiotropy theory* states that the same late-acting deleterious genes seen above, could rather be involved in a positive selection, for their potential early benefits during life, provided that they would only provoke harmful side effects enough, with fitness reduction and ageing, at later ages (Williams 1957).

Note that these evolutionary theories are not mutually exclusive and therefore both may operate at the same time. The actual relative role of each evolutionary mechanism to ageing has not yet been determined. They have also a significant limitation: they are almost exclusively applicable only to sexually reproducing organisms even though ageing is a very general phenomenon in nature.

The other important category embraces the **chemical hypothesis**, which explains ageing by a somatic process deterioration that results from cumulative damage to biomolecules and increased molecular disorder. It is primarily a stochastic process that occurs systemically before and after reproductive maturity and is not defined by any genetic predestination. This loss of molecular reliability ultimately will exceed repair and turnover capacity with an increased vulnerability to ageing or age-associated diseases (Hayflick 2007).

Several theories emerged within the chemical hypothesis:

The error-catastrophe theory suggests that time-dependent errors seen in the machinery for replication, repair, transcription and translation of genetic information will eventually result in cumulative errors in critical enzymes, such as DNA and RNA polymerases or enzymes involved in protein synthesis and turnover. This proliferation of mistakes and resultant accumulation of dysfunctional macromolecules will lead eventually to ageing processes and homeostasis collapse (Orgel 1963). Consistent with this theory, it is known that an increasing amount of denatured, or modified, functionally inactive enzymes do accumulate in cells (with likely pathologic abilities) as a function of ageing (Bloom 2014).

The *disposable soma theory* states that ageing and deterioration are basically a trade-off for increased reproductive fitness during youth. The premise is that the high investment in self-repair is a disadvantage since it consumes precious energy resources (among others) that would be better used for reproduction and caring for the offspring. This compromises somatic repair systems. The result is that ageing will occur through the gradual accumulation of these unrepaired somatic defects – disposable soma – with the level of maintenance being set for avoiding deleterious effects until the reproductive age (Kirkwood 1977).

More general chemical theories portrait ageing as the result of chronic, cumulative chemical (non-enzymatic) modification, insults or damage to all biomolecules – see below *The Mechanisms of Ageing*. This damage, due to endogenous or exogenous agents, is most apparent in long-lived tissue proteins with low turn-over rate.

Some authors attempted a general theoretical framework that would integrate the success of evolutionary and chemical theories of ageing with the observational and experimental data. One of these efforts explored the general theory of systems failure known as *Reliability Theory* (Gavrilov and Gavrilova 1991, Gavrilov and Gavrilova 2003). In this hypothesis, *Gavrilov et al.* proposed that there may be no specific underlying elementary "ageing process itself" – instead it may be an

intrinsically property of a *redundant* system. Systems, which are redundant in numbers of irreplaceable elements, do deteriorate and fail over time, even if they are made of “non-ageing” elements. *Failure rates*, like death rates, should increase with age according to the physical laws that explain kinetics failure of mechanical devices. However, biological systems seems to fail according to the Gompertz-Makeham law of mortality for which there is an exponential increase of the failure rates with age (Gompertz 1825, Makeham 1860).

In humans, this law of mortality describes rather accurately the age dynamics of mortality between the ages of 30 to 80 years. Yet, above this cut-point, studies have found that the expected increase of death rates starts to slowdown and becomes unexpectedly stable – a phenomenon known as the *late-life mortality deceleration* (Gavrilov and Gavrilova 2011, Barbi, Lagona et al. 2018). This surprizing decline in failure rates at advanced ages observed in humans is the same phenomenon of 'almost non-aging' survival dynamics, recognized since the 1970s, and seen at extreme old ages of many other biological species including rodents and invertebrates (Economos 1979). These observations of exponential failure rate and apparently paradoxical "no aging" at extreme ages have led to the search for other mechanisms underlying biological ageing. In living organisms, the reliability of the system is achieved not by the high initial “quality” but also by the number of elements present and the process of forming *de novo* large numbers of new biomolecules or cells (*redundancy*). During our life span, as we lose a big part of this renewal capacity, redundancy depletion will start (especially for irreplaceable elements) (Gavrilov and Gavrilova 2003). Damage tolerance and this loss of redundancy could help explain the observed ‘compensation law of mortality’ as well as the mortality convergence and deceleration seen at older ages. Another attempt to explain the exponential deterioration of biosystems led to the idea that biological organisms start their adult life already with a *high load of initial damage* (Gavrilov and Gavrilova 2001). This prediction is supported by some experimental evidence of early programming of ageing and longevity through the level of initial damage (Vaiserman 2014, Preston, Reynolds et al. 2018).

A recent approach to senescence of biological systems was the addition of *the Chaos Theory* (Poincaré 1890, Lorenz 1963). The presence of chaos in physiological systems was firstly proposed by Mackey *et al.* decades ago after recognising that various biological systems present non-linear dynamics. (Mackey and Glass 1977). The endocrine and cardio-vascular system are standard examples of non-linear dynamics (Dokoumetzidis, Iliadis et al. 2002, Perkiomaki, Makikallio et al. 2005, Hendrix, Hughes et al. 2014). The progressive loss of complexity and the magnification of chaos could lead to an unhealthy and, sometimes, aged system (Lipsitz and Goldberger 1992).

Studies about ageing and chaos dynamics with fractal physiology and early definitions of outcome are still scarce but reveal a promising field for future research (Goldberger, Amaral et al. 2002, Chen and Pham 2013, Zaia, Maponi et al. 2017).

The mechanisms of ageing

It has been considered that control of ageing and longevity are probably incidental to the genome's main goal. Genes seem to drive not the ageing process *per se* but to govern the levels of excess physiological capacity, repair, and turnover – that will indirectly determine longevity. Conserved gene-regulatory pathways coordinate separate processes of cellular health. Because cellular performance is controlled across a wide range of systems, the processes of regulating it are commonly linked. For example, poor protein processing leads to defective function, defective function may increase exposure to reactive oxygen species, and these will cause further low protein quality (see below). Each of these processes is connected to regulation of ageing at the cellular level, and will also ultimately impose their defects on the whole organism (DiLoreto and Murphy 2015).

Recently, *Lopez-Otin et al.* suggested nine common denominators of ageing based on cellular and molecular categories (Lopez-Otin, Blasco et al. 2013). All these hallmarks manifest during normal ageing and appear to coordinate senescence:

1. Genomic instability
2. Telomere attrition
3. Epigenetic alterations
4. Loss of proteostasis
5. Deregulated nutrient sensing
6. Mitochondrial dysfunction
7. Cellular senescence
8. Stem cell exhaustion
9. Altered intercellular communication

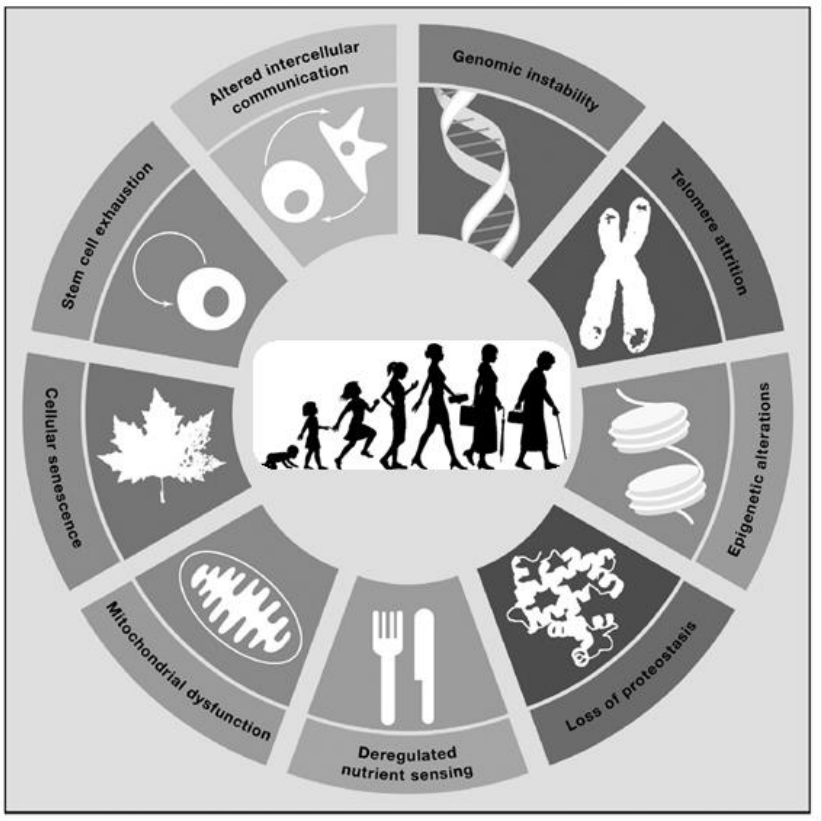

Figure 1.1. The nine hallmarks of ageing. Adapted from *Lopez-Otin, Blasco et al.* 2013.

Genomic instability. There is extensive experimental evidence that somatic mutations, other forms of DNA damage (like chromosomal aneuploidies and copy-number variations) and/or defects in nuclear architecture, do accumulate within cells from aged humans (Faggioli, Wang et al. 2012, Baker, Dawlaty et al. 2013, Moskalev, Shaposhnikov et al. 2013). Studies in mice and in humans with deficiencies in DNA repair mechanisms have shown accelerated ageing in rodents and contribute to several human progeroid syndromes, such as the *Werner* and *Bloom* syndromes (OMIM #277700 and #210900, respectively) (Epstein, Martin et al. 1966, Hoeijmakers 2009). Mutations and deletions in mitochondrial DNA have also been associated to ageing (Park and Larsson, 2011). Defects in the nuclear lamins (responsible for architecture of chromatin and protein complexes that regulate genomic stability) were also found in accelerated human ageing syndromes (*e.g. Hutchinson–Gilford* Progeria Syndrome, OMIM #176670) and progeria animal models (Hutchinson 1886, Eriksson, Brown et al. 2003).

Telomere attrition, a unique form of DNA damage that reaches telomeres, a repetitive sequence found at the end of chromosomes, with high sensibility to age-induced decline (Blackburn, Greider

et al. 2006). At the extreme ends of the chromosomes, DNA synthesis is restricted due to the lack of sequences further upstream for DNA primase engagement. Therefore, each cycle of chromosome replication will necessarily result in telomere shortening. The capacity to replicate the terminal ends of linear DNA molecules in mammals is restricted to a specialized DNA polymerase – telomerase. Its function is to maintain the length of telomeres at the 3′-end of chromosomes and is only found in foetal tissues and in adult stem or tumour cells. This lack of telomerase in most somatic cells will lead to the wearing-off of telomere-protective sequences from chromosome ends. This shortening of telomeres is closely linked to the *Hayflick limit* (the proliferative capacity limit observed in some *in-vitro* cultured cells) and to ageing (Hayflick and Moorhead 1961, Blasco 2007). Telomerase deficiency and short telomeres are also associated to some premature ageing diseases and to increased mortality rates in young humans (Boonekamp, Simons et al. 2013). Also relevant to this telomere attrition concept is the observation that cancer cells, which are "immortal", maintain an active telomerase activity indefinitely (Bernardes de Jesus and Blasco 2013).

Epigenetic alterations. Genetic factors, besides gender, account for about one third of variation of human longevity with currently more than thirty candidate genes proposed (*e.g. APOE*, *FOXO3A*, *IGF1*-R, *CETP*, among others) (Wheeler and Kim 2011). Epigenetics defines genomic modifications that result in deviations of gene expression and phenotype without a matching alteration in DNA sequence. Multiple systems secure the generation and conservation of epigenetic patterns such as DNA methyltransferases, histone acetylases and deacetylases, methylases and demethylases, protein complexes implicated in chromatin remodelling and non-coding RNAs (e.g. miRNAs). DNA methylation occurs mainly as 5-methylcytosine in CpG dinucleotides islands present in most human gene promotors (Caiafa and Zampieri 2005). Hypermethylation of such islands has been linked to transcriptional silencing of the associated genes but its role in the ageing process remains unclear, with some age-dependent global hypomethylation and simultaneous rates of hypermethylation being described in several genetic *loci* during senesce (Schumacher, van der Pluijm et al. 2008). (Maegawa, Hinkal et al. 2010). Chromatin remodelling by histone modifications and methylation has also been found associated to senescence. One important example are sirtuins, a class of NAD-dependent proteins that possess either mono-ADP-ribosyltransferase or deacylase activities (Yamamoto, Schoonjans et al. 2007). They have been extensively studied in numerous aspects of ageing. Various authors have found that, in mammals, at least three members of the sirtuin family (*SIRT1*, *3* and *6*) are caught up in normal ageing by improving genomic stability and metabolic efficiency (Oberdoerffer and Sinclair 2007, Someya, Yu et al. 2010, Kanfi, Naiman

et al. 2012). Transcriptional dysfunction also appears to be present in human senesce (Harries, Hernandez et al. 2011). Age-related aberrant production of non-coding RNAs may have an impact on lifespan trough targeting longevity-linked systems and regulating stem cell performance (Ugalde, Espanol et al. 2011). Several studies have confirmed the role of various of these miRNAs on active modulation of ageing in animal models (Liu, Landreh et al. 2012).

Loss of proteostasis. Ageing is associated with the accumulation of damaged macromolecules, especially proteins. Proteostasis refers to the capacity for the stabilization of correctly folded proteins and for the normal removal of degraded proteins by the proteasome or the lysosome (Powers, Morimoto et al. 2009). Chronic accumulation of damaged and aggregated proteins occurs in many tissues during ageing in several age-related chronic diseases, such as Alzheimer's and Parkinson's diseases as well as in eye cataracts (Rubinsztein, Marino et al. 2011). There are many examples linking declining protein clearance with ageing through studies evaluating disturbed proteolytic pathways (autophagy-lysosomal or ubuiquitin-proteasome) or intervention therapies that are inducers of autophagy, like the mTOR inhibitor rapamycin (Cuervo 2008, Wilkinson, Burmeister et al. 2012).

Deregulated nutrient sensing. Anabolic and nutrient sensing signalling pathways dysfunction are associated with ageing. In mammals, glucose sensing somatotrophic axis and insulin-IGF1 system share downstream intracellular targets, such as the mTOR complexes and FOXO transcription family, that have been linked to longevity (Barzilai, Huffman et al. 2012). Modulation of this bioenergetic pathway through dietary restriction has demonstrated a significant positive effect in the lifespan of several study models (Lin, Kaeberlein et al. 2002, Colman, Anderson et al. 2009). Other nutrient-sensing systems like AMPK and sirtuins, by detecting intracellular AMP and NAD levels, respectively, seem also to have an important role in ageing. By determining intracellular nutrient shortage they will trigger a unified response that connects low-energy states with genomic translation pathways that promote longevity (Fontana, Partridge et al. 2010). Several studies have demonstrated that pharmacological inhibition of mTOR by rapamycin (not chronically) can simulate nutrient scarcity in cells with positive lifespan effects (Harrison, Strong et al. 2009, Neff, Flores-Dominguez et al. 2013).(Harrison, Strong et al. 2009, Neff, Flores-Dominguez et al. 2013).

Mitochondrial dysfunction. Mitochondria are of crucial importance for multiple cellular processes such as ATP production (through phosphorylation of ADP via the respiratory chain), beta-oxidation of fatty acids and apoptosis. The relationship between mitochondrial function deterioration,

respiratory chain dysfunction and ageing has been extensively studied but its specifics are not fully understood. The free radical theory of ageing was first introduced in 1956 by Harman (Harman 1956). The premise states that highly reactive oxygen-derived substances (free radicals), coming mainly from the mitochondrial respiratory chain, would result in accumulation of macromolecular and cellular damage. It was postulated that these reactive species would act as a cellular signal for ageing and that their tissue levels would eventually determine overall lifespan of the organism. Some proof of concept was found when several authors unravelled the effects of reactive oxygen species on DNA, lipids and proteins, and the inverse correlation between basal metabolic rate (rate of oxygen consumption per unit weight) and maximum lifespan of mammals (Speakman, Selman et al. 2002, Bokov, Chaudhuri et al. 2004). Despite these data, recent publications with animal models genetically modified to increased antioxidant defences and intervention studies with administration of antioxidant drugs did not show any true benefit on extending longevity (Perez, Van Remmen et al. 2009, Ristow and Schmeisser 2011, Kauppila, Kauppila et al. 2017). Today, it is believed that beneath a certain cut-off point, reactive oxygen species may rather serve as a survival-signal (like the ones observed in nutrient depletion – see above) and promote compensatory cell responses with downstream increased life span (Hekimi, Lapointe et al. 2011, Ristow and Schmeisser 2011). Above this threshold, they will become deleterious, resulting in longevity reduction. Interestingly, some other mild toxic substances may also trigger advantageous mitochondrial responses that will overpass the actual damage that they may provoke – a concept denominated as mitochondrial hormesis. Metformin and revestrol are known examples of this idea (Lagouge, Argmann et al. 2006, Onken and Driscoll 2010). During the last portion of lifespan, organisms' ability to neutralize, repair or remove reactive oxygen species and the resulting induced damage on cellular components is weakened. This decrease in clearance efficiency will be further intensified during ageing and ultimately culminate in cellular and organisms' death." (Garaschuk, Semchyshyn et al. 2018). Despite all these somehow controversial theories about how mitochondria dysfunction influences lifespan, today there is consensus about the profound effect that these intracellular organelles have on the overall ageing process (Kujoth, Hiona et al. 2005, Lee and Wei 2012, Zhang, Menzies et al. 2018).

Cellular senescence. This concept was first introduced by *Hayflick* in human fibroblasts and represents a steady arrest of the cell division cycle coupled with some phenotypic modifications (see above – the *Hayflick* limit and *telomere attrition*) (Kuilman, Michaloglou et al. 2010). It is currently accepted that many ageing mediators may trigger cellular senescence outside telomeric

deterioration. These include over 50 oncogenic and mitogenic DNA alterations able to induce cellular senescence *per se* (Gorgoulis and Halazonetis 2010). The mechanisms involved in these paths are not fully understood but, by inducing senescence, they are likely to prevent damaged cell propagation through natural clearance by the immune system. Therefore, supposedly the concept of cellular senescence is rather a beneficial one – to stop dysfunctional cell proliferation. Recent data have revealed that increased presence of ageing cells in old tissues (and individuals) is not a consequence of an augmented global senescence but rather a lack of local efficient cell replacement system (by low clearance, low cell regeneration or both) (Hoenicke and Zender 2012, Rando and Chang 2012). Consistent with this concept, several studies have shown that moderate enhancement of senescence-inducing tumour suppressor pathways, like the INK4a/ARF locus, do increase longevity (Matheu, Maraver et al. 2009).

Stem cell exhaustion. The reduction and dysfunction of the stem cell regenerative potential in living tissues is probably an integrative consequence of multiple senescence-associated processes (Flores, Cayuela et al. 2005, Rossi, Bryder et al. 2007). Deficient proliferation of stem cells during ageing has been linked to immune and hematopoietic-senescence and increased incidence of cancer and anaemia (Shaw, Joshi et al. 2010). During post-reproductive maturity, these altered stem cells will achieve tissue supremacy (clone dominance) by exploiting the aged environment and accumulate with age (Goodell and Rando 2015). This clone collapse will not be the cause of ageing *per se*. Organism and tissue senescence will rather be a consequence of the acquired functional deficiencies that these dominant cellular phenotypes will present. Recent studies have suggested that stem cell rejuvenation and transplantation may reverse ageing phenotypes (Harrison, Strong et al. 2009, Rando and Wyss-Coray 2014).

Altered intercellular communication. An important imbalance between pro- and anti-inflammatory cytokines is observed during ageing (Franceschi, Garagnani et al. 2017). This *inflammageing* phenotype may be a consequence of various mechanisms that include the propensity of senescent cells to produce proinflammatory cytokines (IL-6, IL-18), a deficient immune system with diminished pathogen clearance capacities, the gathering of proinflammatory tissue damage, the defective autophagy response and the dysfunction of neuro-hormonal responses (like the case of the ineffective control of excessive inflammatory by the aged Hypothalamus-pituitary-adrenal (HPA) axis – see *Hypothalamus-pituitary-adrenal axis*) (Deeks 2011, Lee, Ward et al. 2012, Salminen, Kaarniranta et al. 2012). However, human studies evaluating centenarians, have shown that these individuals have a prominent pro-inflammatory cytokine that is paralleled by an even higher blood

levels of potent anti-inflammatory molecules, such as TGF-β or cortisol (Franceschi, Capri et al. 2007). Therefore, senescence seems to depend more on the proper balance between pro- and anti-inflammatory factors than on the sole reduction in the level of proinflammatory cytokines (Garaschuk, Semchyshyn et al. 2018). Another example of intercellular communication is contagious ageing, where senescent cells can encourage senescence in the adjacent cells, probably by sharing reactive oxygen species and/or damaged molecules via cell gap-junctions (Nelson, Wordsworth et al. 2012). Conversely, it was also found that lifespan enhancing therapies involving just one single tissue could have retarding effects on the overall organism's ageing course, compelling further studies to elucidate this rejuvenation intercellular communication network (Lavasani, Robinson et al. 2012). An emerging approach has explored the help of molecules circulating in the blood to limit or reverse aspects of ageing in several animal models. In some of these studies, aged animals exposed to young blood through heterochronic parabiosis were able to improve their stem cell function in muscle, liver and brain; as well as to increase dendritic spine density and synaptic plasticity with a parallel recovery in age-related cognitive impairments (Villeda, Plambeck et al. 2014, Castellano 2019).

With this global view of the mechanics of ageing through the biological systems, and its inevitability, we should now review the human quest on how to achieve ageing with maximal health and minimum senescence.

How healthy can ageing be?

1.2 Healthy ageing

Epidemiology of ageing

In the last century, we have witnessed the rise of worldwide human longevity as one of the major achievements of public health and health care. In 2017, there was an estimated 962 million people aged 60-years or over in the world, covering around 13% of the global population (United Nations 2017). By 2050, this number is expected to double (United Nations 2017). In the next years, for the first time in history, the total amount of people over 65-years will outnumber children under age 5 (WHO, National Institute on Aging et al. 2011). This remarkable phenomenon lays on the continuing worldwide increase in life expectancy but especially on the simultaneous falling fertility rates.

Currently, in Portugal, life expectancy at birth has been rising almost 4-yr per decade (OECD 2015). Latest national data puts life expectancy at birth, in 2016, at 83.4-years for women and 77.7-years for men (PORDATA 2018). The estimated proportion of population 65-yr or older, in 2017, was 21.1%, with a life expectancy at 65-years of plus 20.8-yr for women and 17.6-yr for men (INE 2017, PORDATA 2018). At the same time, population under 15-years has been reducing ~2% per decade comprising today only 14.0% of the Portuguese population (Eurostat 2018).

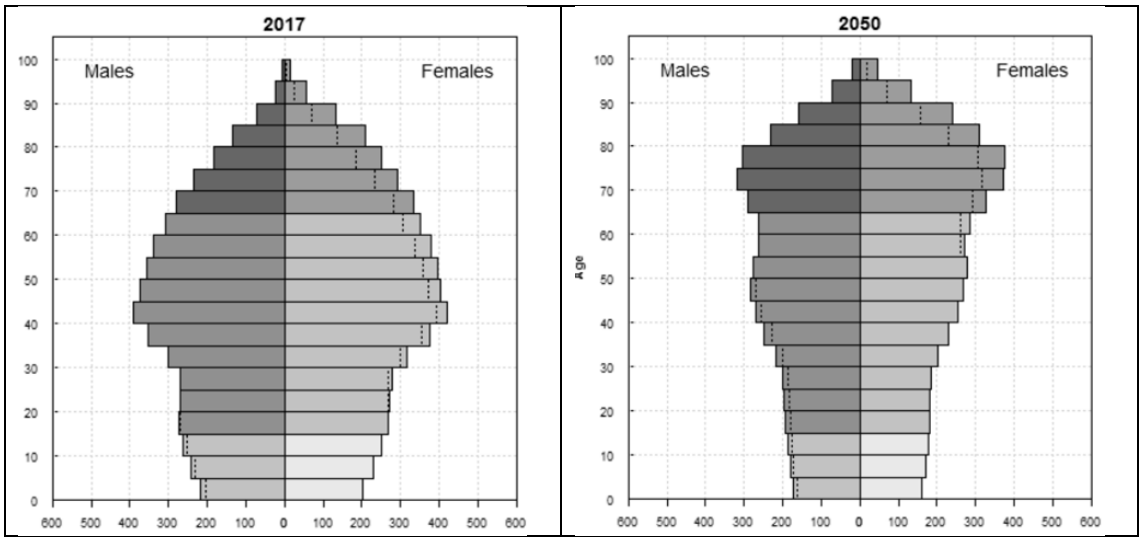

Figure 1.2. Portuguese population age pyramid in 2017 and projection for 2050 (in thousands). The dotted line indicates the excess male or female population in certain age groups. The data are in thousands or millions and represent the population in each age group. Source: United Nations, Department of Economic and Social Affairs, Population Division (2017). World Population Prospects: The 2017 Revision.

Figure 1.3. Total population by age group in Portugal. Source: United Nations, Department of Economic and Social Affairs, Population Division (2017). World Population Prospects: The 2017 Revision.

From 2000 onwards, much of the increase in life expectancy occurred in people over 65-years of age. This progressive ageing of older population itself has been growing at a faster pace than any other age segment of the EU's population, due to mortality rates fall of the very old (OECD 2015). This new demographic of death has a big effect in the age pyramid pushing today the share of those aged 80 years or older (in the EU-28's) above 5%, with a projection to more than double by 2080 (Eurostat 2018). Some authors have proclaimed that life expectancy is close to an ultimate ceiling (Dong, Milholland et al. 2016). Even if there is a natural limit to human lifespan (not pre-designed but nevertheless real) the total number of centenarians will continue to increase, raising many social implications and important questions about the mechanisms of increased longevity and healthy ageing at very-old ages.

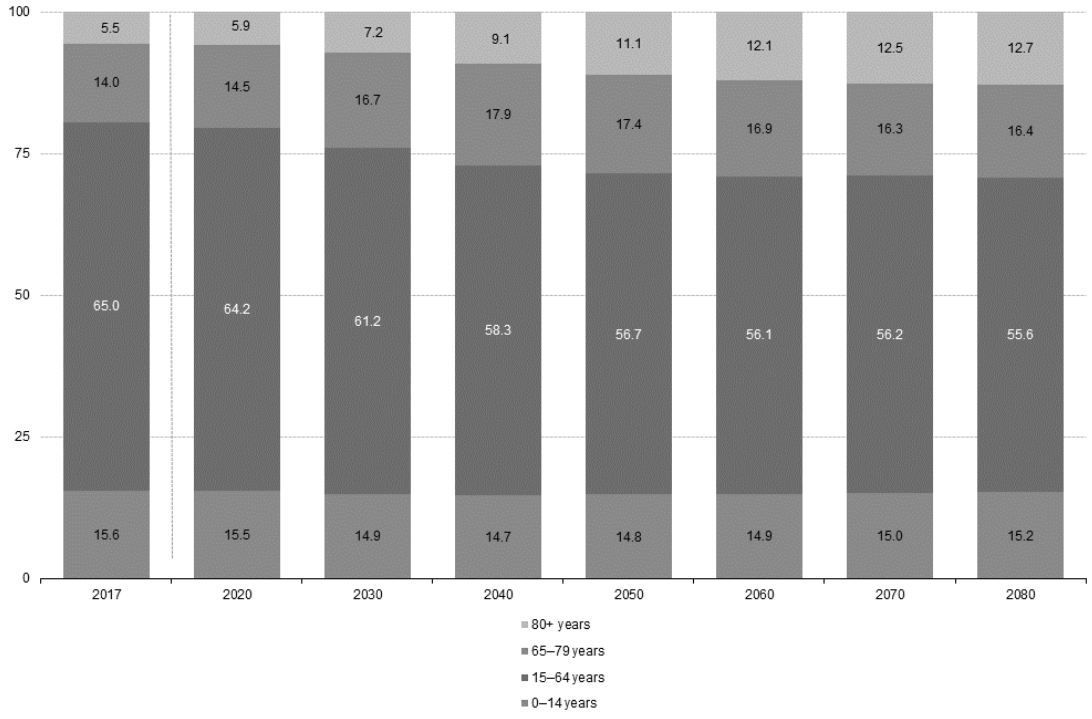

Figure 1.4. Population structure by major age groups in the European Union (EU-28), 2017-2080 (% of total population). Source Eurostat 2018.

Important issues have been created by this demographic change. Individual and social burden of increased longevity makes pressure in health systems. By some estimates, 35% of total medical costs in USA are spent on patients older than 65 years. *Per capita* healthcare costs are three times higher in patients older than 85 years versus those younger than 65 (Keehan, Stone et al. 2017).

As life expectancy hits a barrier it was hypothesized that during the last years of life there would be increased morbidity, with consequent increases in health care costs for seniors - compression morbidity (Fries 1980, Crimmins and Beltran-Sanchez 2011). Compression of disability, rather than of morbidity, is common among centenarians, with over 90% having a history of being functionally independent when they were 93 years-old (Hitt, Young-Xu et al. 1999). However, most people who survive this age essentially compress the time with disease and disability toward the relative ends of their lives (Andersen, Sebastiani et al. 2012).

Unfortunately, although there is strong evidence that older people are living longer, particularly in high-income countries, the quality of life during these extra years is not so obvious (Crimmins and Beltran-Sanchez 2011). In Portugal, despite these longevity increases, after 65 years of age women can expect to live only a quarter of their remaining years without disabilities, while men can expect to live almost two fifths (38%) of those years in good health (Eurostat 2018). While some of this may reflect measurement problems and past deprivation levels affecting our country and this age group, the reduced outcome compared to other European countries (ranking 21th in 25) should be a matter of concern (Eurostat 2018).

This perceived individual (or overall) compressed burden and social/health-care load has fuelled much of the ageing research during the last two decades in search for the "elixir of life" and healthy ageing.

Successful, Healthy and Active ageing

In 1980's one important question behind the current demographic revolution was already recognized (Fries 1980). Life expectancy in itself does not equate with health, and the extra years of life are ought to be healthy ones. Therefore, it was necessary to conceptualise how ageing should be pursued during later years.

Successful ageing is an unspoken expectation in all human cultures but a theoretical construct for this idea was only introduced by *Rowe and Kahn* in 1987 (Rowe and Kahn 1987). It was based on 3 principles: low probability of disease and disease-related disability, high cognitive and physical functional capacity, and active engagement with life. Despite no current consensus for its use and definition, successful ageing concept has helped shape the view of health-span at the individual and society level and re-orientate gerontology research (Rowe and Kahn 2015).

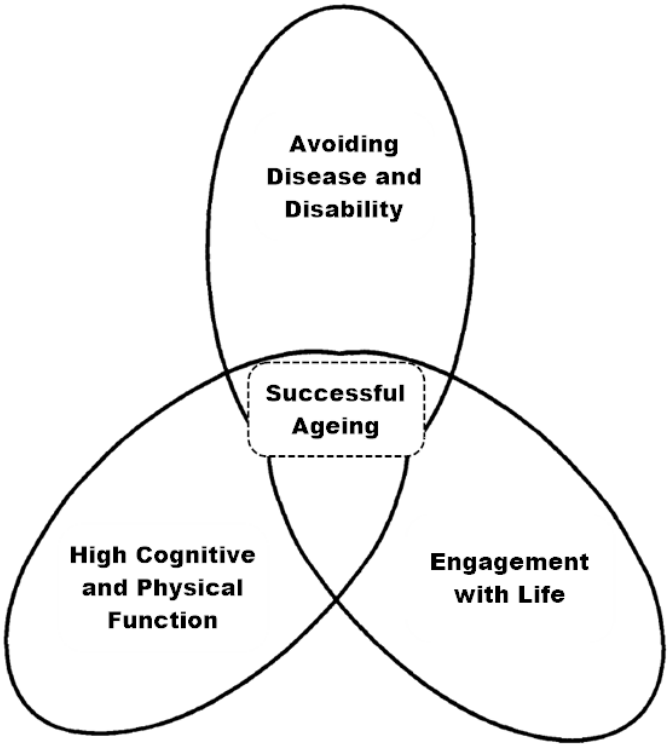

Figure 1.5. The model of successful aging by Rowe and Kahn. Adapted from *Rowe and Kahn 1997*.

Normal ageing, even in the absence of significant comorbidity, is marked by gradual deterioration of many physiological functions (see below). Healthy physiologic function is characterized by an intricate collaboration between multiple control mechanisms that enable the individual to adapt to everyday life challenges (Lipsitz and Goldberger 1992). Healthy ageing is often used to identify a positive disease-free state that distinguishes between healthy and unhealthy individuals. This is inappropriate since many individuals may suffer from one or more health conditions and still be able to cope properly to normal everyday life events. Recognizing this fact, the WHO proposed a more holistic view of *Healthy Ageing* and defined it "as the process of developing and maintaining the functional ability that enables well-being in older age" (WHO 2015). This concept allows that individuals that present some progressive age impairment (with loss of dynamic range in physiologic function), but still capable to be and do what they have reason to value, to be labelled as healthy. The functional ability they have is made up of their intrinsic capacity (both mentally and physically) but also of interactions with relevant environmental characteristics. Most people show

deterioration with age in all physiological systems. However, this effect of ageing is different in every person and may not be apparent until either the subject faces an overwhelming challenge, or some physiological function falls to a critical level. This may result from the great individual reserve capacity or redundancy of some physiological systems and from personal resilience (the ability to maintain or improve a level of functional capacity in face of adversity) (WHO 2015). This exclusive ageing dynamic profile turns each one of us unique.

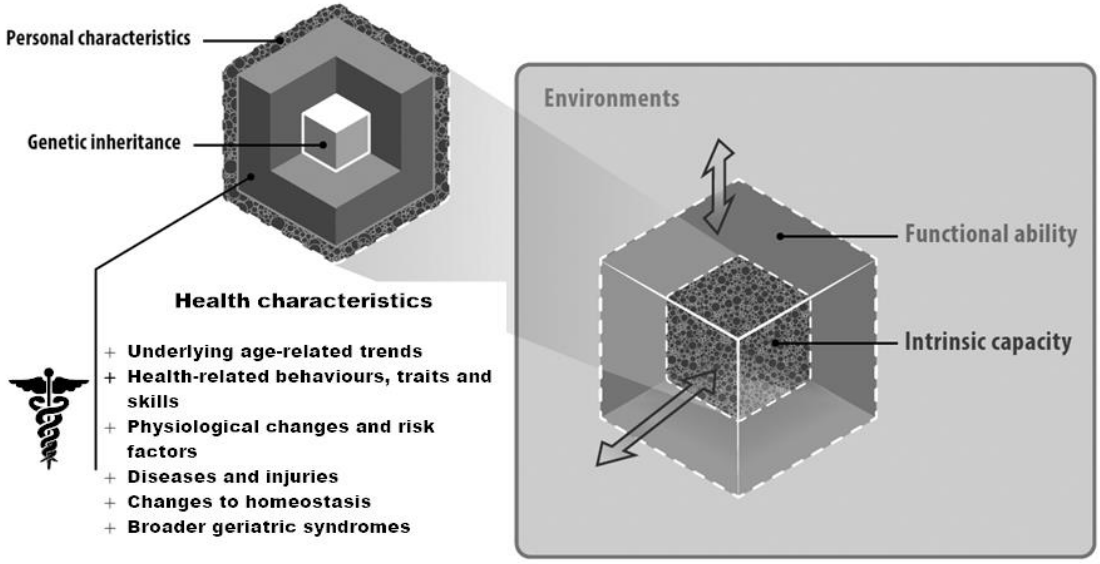

Figure 1.6. Healthy ageing determinants and characteristics. Adapted from: World report on ageing and health, WHO 2015.

Active ageing is a complex concept introduced by the WHO in the late 1990's. It was defined as the process of optimizing opportunities for health, participation and security in order to enhance quality of life as people age (WHO 2002). It was intended to bear a more inclusive message than "healthy ageing" and to recognize other factors, besides health care, that can affect how populations and individuals age (Kalachea and Kickbusch 1997). The individual and environmental factors (such as the economic determinants, physical environment and health and social services) and their role in shaping the ageing course were included in this definition. To note, not all these factors are independent but rather an entwined environment that will shape an individual senescence phenotype.

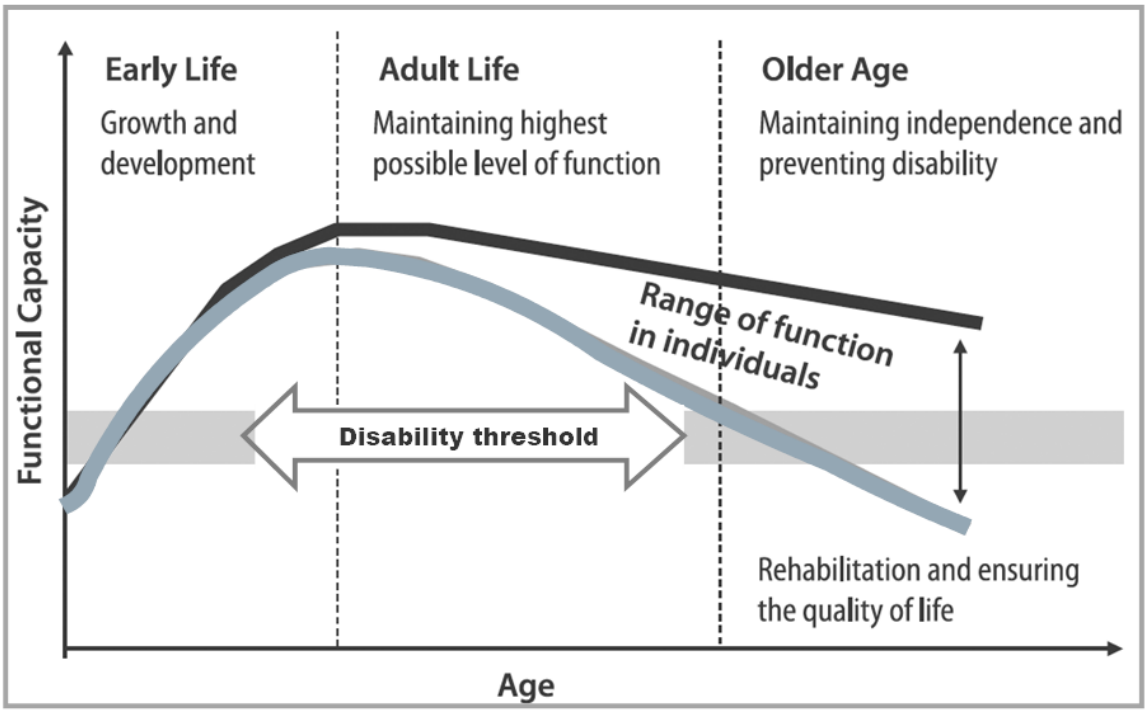

Figure 1.7. Maintaining functional capacity over the life course. Changes during lifetime may lower the disability threshold, thus decreasing the number of disabled people in each community. Adapted from the Active Ageing: A Policy Framework, WHO 2002.

Based on these assumptions and considerations, the question that follows pertains on how to measure healthy ageing years? Life expectancy in itself does not address whether longer life is experienced in good health. To tackle this issue some population indicators were created with the concept of *health expectancy* (Robine and Ritchie 1991). Since these are population health indicators that combine information on the quantity of life (life expectancy) and quality of ageing years, there are many possible health expectancies as health dimensions. The most common practice is to use self-reported general health (*healthy life expectancy*) and disability (*disability-free life expectancy*). Unlike *quality-adjusted life-years* (QALYs – combination number that measures health as a function of length of life and quality of life), health expectancies do not generally incorporate health states categories and tend to be a more "real" metric of good years in healthy condition (Sassi 2006, Beresniak, Medina-Lara et al. 2015). More recently, a harmonized health measurement for European countries was developed. *Healthy life years* (HLYs), a disability-free life expectancy measure, that allows comparisons between different EU nations. It is based on limitations in daily activities and therefore evaluates the number of remaining years that a person of a certain age can expect to live without disability (Lievre, Jusot et al. 2007). This indicator,

computed annually across all EU countries, highlights the enormous differences across Europe and how common life expectancy metrics may mask inequalities (Jagger, Gillies et al. 2008). Last published data reveals that for Portugal in 2015, despite slightly better life expectancy than the EU-28 average, women and men at age 65 could expect to spend, respectively, only 25% (5.4-years) and 39% (7.0-years) of their remaining future life without reporting long-term activity limitations. Average EU-28 was 9.4-years (EHLEIS 2018, Eurostat 2018).

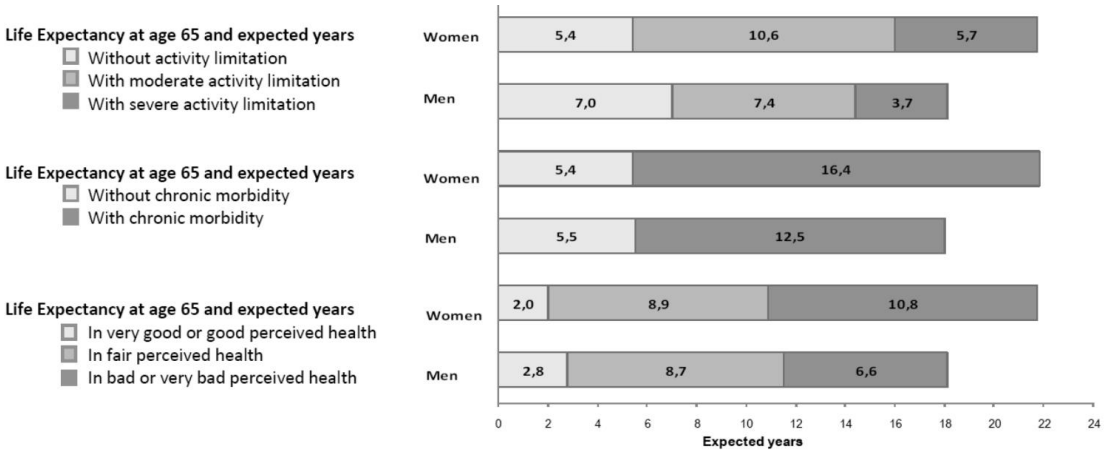

Figure 1.8. Portuguese life and health expectancies at age 65 based on activity limitation (HLYs), chronic morbidity and perceived health. Source: EHLEIS Technical report 2018_4.1, May 2018; European Health and Life Expectancy information system.

Several factors can explain this high prevalence of future "unhealthy" ageing at 65-years of age, including early deterioration of the internal physiological systems. Although the nature of the ageing process is similar in all humans, important quantitative differences between individuals exist. Some, with great reserve capacity or redundancy of physiological systems, will not present senescence until later in life. The clock of ageing will catch others much earlier.

Several common ageing effects on the different human organ/systems are briefly summarized, as follows:

Cardiovascular system. Lack of elasticity with increased stiffness of blood vessels and progressive atherosclerosis are the main effects present during ageing. The amplified severity of blood turbulence will provide a catalyser for more atherosclerotic lesions and augmented inflammation. Arteries also present diminished response to vasodilatation or constriction. Common cardiac

changes associated with ageing are: diastolic dysfunction, ventricular septal thickness, reduced number of pacemaker cells, increased dysrhythmia, etc. In general, all cardiovascular system loose stress compliance (Akhtar 2017).

Respiratory system. Three major age-related changes occur in the respiratory system. First, loss of elasticity, both in the lung and in the chest wall, will reduce pulmonary vital capacity, increase residual volume, and therefore amplify the work of breathing and the susceptibility to respiratory failure. Second, reduction of alveolar gaseous changes with the drop of mean arterial oxygen tension. Third, diminished central respiratory drive to hypoxia and hypercapnia (Akhtar 2017).

Renal system. A progressive decline in the glomerular filtration rate is observed during senescence (about 1mL/min per year after the 3rd decade of life). Some tubular dysfunction is also detected with diminished ability to excrete and reabsorb solutes. Both these changes and a decreased in renal medullary tonicity, will increase the risk of hypo-osmolar states commonly seen in aged individuals. Elderly are incapable to maximally concentrate or dilute urine. Their sensitivity to plasma volume or osmole changes is also impaired which result in a dysfunctional thirst response. Attenuation of the autonomic renal vascular response is frequently present and predispose the aged kidney to increase damage from hypo- or hypertensive states (Akhtar 2017).

Gastrointestinal system. During ageing the most frequent gastrointestinal disturbance observed is abnormal motility of the oropharyngeal/upper oesophageal and colonic area. No significant changes are noted in liver function tests (Akhtar 2017).

Immune system. A low-grade chronic inflammation state is frequently seen in the elderly. This is consistent with the increased balance towards pro-inflammatory cytokines observed during senescence. Age-related functional changes are also present in T-cell and B-cell functions with a decrease in bactericidal activity of immune cells and non-cellular immune mechanisms. These features are thought to be connected to the increased frequency of infection and cancer found in the elder (Akhtar 2017).

Table 1.1. Decline in biochemical and physiologic systems of vertebrates during ageing [adapted from *John W. Baynes Medical Biochemistry*, Chapter 43, 592-602; 4th Edition, 2014, Elsevier (Baynes 2014)].

Biochemical	Physiologic
• Basal metabolic rate	• Cardiovascular performance
• Glucose tolerance	• Lung expansion volume
• Protein turnover	• Renal filtration capacity
• Oxidative phosphorylation	• Nerve conduction velocity
	• Immunological defences
	• Musculoskeletal system strength

Sarcopenia and body composition. Older people tend to lose weight during ageing. This usually represents a loss of lean body mass but also of total fat content, despite an increase of percentage of fat per total body weight. Visceral fat increases and accumulates also in muscle. The loss of muscle quality and mass translates into reduced muscle strength and functioning. Bone metabolism is significantly affected with ageing with progressive loss of bone mass and plasticity with reabsorption exceeding formation starting as early as the third decade of life (Masoro 2016, Akhtar 2017).

Central nervous system. Several aspects of the central nervous system function are significantly affected during ageing. *Sensory function loss*. Most sensory systems display some decline with age. Among aged people, some degree of hearing loss is almost always present, particularly of high-frequency sound. This can make it hard to distinguish spoken words from the background noise which increases social isolation in some individuals. Vision is also commonly affected during senescence. During ageing a progressive deterioration of the accommodation power is observed, presbyopia is common, as is the reduction of retinal cones and the loss of adaptation of retinal rods to low intensity light. Together with the decreasing in pupil size adaptation to light, vision in the elderly is less suited to darkness or intense light. Some other age-related diseases may also increase visual disfunction, such as cataracts, glaucoma and macular degeneration. Along with ageing there is also the decline in olfaction and taste sensory abilities (Akhtar 2017).

Motor function modifications. A common effect of ageing is the increased time of reaction and a slower motor response. Deterioration of the sensory system, decreased central nervous processing and the decline in muscle strength, are all factors at the origin of this age-dependent dysfunction.

The result is many times a slower, and somewhat less coordinated, movement. Not surprisingly, elderly people frequently present some difficulties in maintaining posture and balance with an increased risk of falls. Older individuals tend to present osteoarthritis, specially involving the knee and ankle joints, which ads to their reduced motor capacities (Akhtar 2017).

Cognitive function. Many aspects of the cognitive performance change during ageing. They will be more extensively addressed in the next section (see *Ageing and cognition*).

Endocrine system. Similarly, several endocrine organs and functions are influenced by age-dependent mechanisms. A more detailed description of these modifications is provided in a following chapter (see *Some Endocrine perspectives on ageing and cognition*).

1.3 Ageing and Cognition

What is Cognition?

Animal cognition refers to mental abilities involved in perception, attention, thinking, understanding, learning, remembering, solving problems and making decisions (Premack 2007, IOM 2015). It is a multidimensional aptitude that depends on a variety of dissociable capacities that, in humans, include memory, executive function, language and attention. This distinction has functional, neuro-anatomical and methodological relevance.

Ageing and Cognition

The impact of ageing on cognition varies widely from one individual to the other (Paulo, Sampaio et al. 2011, Rapp and Bachevalier 2012). As adults age, their performance on some psychometric tests changes systematically, a result that is widely considered when reporting cognitive decline during normal ageing. Despite neurobiologically similarities within the neurobiological systems involved, distinct cognition dimensions tend to present different vulnerabilities and evolve distinctly over the lifespan (Ramscar, Hendrix et al. 2014). The idea that cognitive deterioration is a natural part of ageing is controversial. This impression has been contradicted by many authors, especially after the first publications on crystallized and fluid intelligence (Horn and Cattell 1966). The crystallized and fluid intelligence theory encompasses two principal components of general intelligence. The ability to perceive relationships, engage in formal reasoning, and understand intellectual and cultural heritage – *crystallized intelligence*; and the "inherent mental ability", product of the brain's information processing system, that includes attention, memory capacity and the speed processing used in thinking and acting – *fluid intelligence* (Jaeggi, Buschkuehl et al. 2008). The environment, education and learning can affect crystallized intelligence. Culture-specific items such as number facility, verbal comprehension and general information usually measure it. Fluid intelligence is largely considered to be independent of education or environment and depends more on the intrinsic legacy of the individual (Jaeggi, Buschkuehl et al. 2008). The items usually used to test fluid intelligence include memory span, inductive reasoning and figural relationships. Because fluid intelligence involves intellectual functions greatest affected by fluctuations in the brain structure status, it has been generally assumed to be the intelligence part of cognition that declines with age (Horn and Cattell 1967, Manard, Carabin et al. 2014). Negative changes seen

here (despite important individual heterogeneity) are the ones mainly dependent on working memory, processing speed and verbal fluency (Park and Gutchess 2002, Davidson, Zacks et al. 2003). Performance in knowledge-dependent verbal abilities, implicit and autobiographical memory seems to be secure until late in life (Spencer and Raz 1995, Ardila 2007). So, the decline that occurs in the healthy elderly may reflect the slowing of central processing rather than the capacity to use information. The trajectory of cognitive changes also varies for distinct cognitive functions and is not associated solely with decline; some aspects of cognition, such as wisdom and knowledge, remain stable or even increase until older decades. Some of these findings can be elucidated on the basis of the *cognitive reserve* hypothesis (Stern 2002). This is defined as the ability to optimize or maximize cognitive performance through differential recruitment of neurophysiological networks and/or alternative cognitive strategies (Stern 2012). It represents a model of brain resilience to age damage of biological structures that support cognition seen by many authors in animal models and in humans (Brown, Cooper-Kuhn et al. 2003, Bartres-Faz and Arenaza-Urquijo 2011, Bennett, Arnold et al. 2014). In terms of the cognitive processes involved, one assumes that the cognitive reserve operates by allowing a more flexible strategy usage of brain/cognitive resources, an aptitude connected to the executive function (Tucker and Stern 2011).

Studies of brain tissue in both humans and in animal models have pursued to examine the underlying neural mechanisms responsible for age-related changes in cognition. These include studies of neuronal number, synaptic integrity and neurotransmitter changes. Overall, they show that neuronal number remains relatively stable, although changes do occur in neuronal structure and neurotransmitter receptors. Contrary to the common idea, it is known today that neuronal loss is not a feature during brain ageing. The stability in the number of neurons in areas supporting cognition is seen in normal senescence (in contrast to the extensive neuron loss that occurs in some age-related neurodegenerative diseases) and is supported by several animal and post-mortem human studies (Amaral 1993, West, Coleman et al. 1994, Boldrini, Fulmore et al. 2018). Regional synaptic number and function changes appears to be relevant to cognitive senescence. Dendritic and spine number reduction and retraction are found in dorsolateral prefrontal cortex and hippocampus with correlation with the observed cognitive decline (Dumitriu, Hao et al. 2010, Morrison and Baxter 2012). Significant changes also occur in neurotransmitter signalling. For instances, the number of glutamate AMPA receptors decrease in the hippocampus (Hara, Punsoni et al. 2012). In combination with thalamic contraction, pre-synaptic and postsynaptic dopaminergic

neurotransmission is affected leading to the impairment of dopaminergic signal transduction pathways (Collier, Lipton et al. 2007). Norepinephrine levels are altered by decreases in the production of neurotransmitters by the basal forebrain and the *locus coeruleus*, while levels of α2-agonist receptors have been shown to decrease (Arnsten, Cai et al. 1988, Robbins and Arnsten 2009). Additionally, in ageing, cholinergic basal forebrain system signalling, which plays a crucial role in learning and memory, can also be impaired with fibre degeneration of affected neurons and their projecting axons (Nyakas, Granic et al. 2011).

There is an overall reduction in brain cellular regenerative capacity. Despite a nonlinear trend in age-related shrinkage, the mass of the brain decreases by approximately 15% after mid-fifties (Raz, Lindenberger et al. 2005). This decrease is due mainly to shrinkage of cell volume, myelin loss and some cell loss. There is a compensatory increase in cerebrospinal fluid volume, expansion of the capillary network and swelling of perivascular spaces (Meier-Ruge, Ulrich et al. 1992). However, not all areas of the brain shrink at the same rate. Some areas (*e.g.* entorhinal cortex) are almost not affected by aging, whereas there is a significant decline in thalamic, cerebellum and cortical grey matter size (Salat, Buckner et al. 2004, Raz, Lindenberger et al. 2005). Macro- and micro-structural white matter also suffer important changes during healthy ageing with an overall reduction of volume and a functional reorganization, especially in the anterior brain regions (Tomasi and Volkow 2012, Marques, Soares et al. 2016).

Based on this organization, neuropsychological research has provided important data about the brain systems that mediate age-cognitive dysfunction and their role in distinct aspects of human cognition.

Memory

Memory is usually thought of as the aptitude to recall past events and learned information. It can be divided into the length of time the information has been stored (short-term memory or long-term memory), their use may be either conscious or unconscious (explicit, declarative vs. implicit, nondeclarative) or be organized by the type of item being kept (visual, verbal or autobiographical data) (Martin and Li 2016). Like other dimensions of cognitive functioning, distinct subtypes of memory diverge in how they change with ageing.

Working memory (short-term memory). Working memory refers to the active maintenance of verbal and nonverbal information in the mind for potential manipulation to complete goal-directed tasks

and behaviours (Matthews 2015). It is an explicit, declarative memory subtype and generally considered a component of executive function (see below). The age-dependent decline of working memory is associated with the level of complexity of the task and the presence of a distraction (Salthouse and Meinz 1995). Although this subtype of memory is an important aspect of the learning process, attention and processing speed are inextricably linked to this skill. During ageing, older adults perform cognitively best when they can focus on one task at a time and do not divide their attention and processing speed. Several studies have shown that working memory uses a network of cortical and subcortical areas, depending on the task executed, but virtually all the time involving the participation of the prefrontal cortex (Rowe, Toni et al. 2000). Functional imaging has proposed that *phonologic* working memory (for processing language information, *e.g.* keeping a number "in your head") involves more areas on the left side of the brain, whereas *spatial* working memory tends to include more regions on the right side (Fletcher and Henson 2001).

Long-term memory. Long-term memory refers to the relatively permanent memory storage (the repository of a person's knowledge) and involves the processes of encoding, consolidation and retrieval of information (IOM 2015). There are two main subtypes of long-term memory: explicit (or declarative) and procedural (implicit) memory.

Explicit memory refers to the intentional recollection of previous experiences and can further be divided into episodic memory and semantic memory (IOM 2015). *Episodic memory* is the capacity to consciously recall autobiographical events, including times, places, associated emotions and other contextual information. This memory subtype is unique because it is particularly related to both a sense of self and of time (Tulving 2001). It tends to decline with age, especially when the task demands are more complex or when there are few environmental cues available (Mitchell 1989). While the hippocampus is considered critical to all memories consolidation, its role after consolidation and in non-episodic memories is still subject of controversy (Matthews 2015). Disruption of the medial limbic circuit (including mamillary bodies, anterior nuclei of the thalamus and fornixes) as well as injury to the posterior cingulate gyrus, which is functionally connected to it, will also impair episodic memory (Chen, Chuah et al. 2010). The frontal lobes are involved in the crucial task of encoding and retrieval tasks of information needed for episodic memory (Maril, Davis et al. 2010). Distinction between verbal and visual episodic memory functions translates into lateralized neuroanatomical organisation – left hippocampal system for verbal encoding and right hippocampal for visual and spatial learning tasks (Chen, Chuah et al. 2010). *Semantic memory* is the ability to consciously recall factual information

acquired over a lifetime, their relationships and uses, including abstract concepts, as well as words and their meanings. This subtype of explicit memory is dissociable from episodic memory; older adults typically perform as well, or better, than young adults do (Spaniol, Madden et al. 2006, Ramscar, Hendrix et al. 2014). During ageing, individuals may have some difficulty in retrieving semantic information that has not been used for some time, but usually the data can be accessed with the proper cues (IOM 2015). The clinical presentation of semantic memory deficits most often involves the cognitive domain of language (Matthews 2015). A wide range of brain areas are involved in semantic memory depending on the data stored (*e.g.* motion, sound, olfaction, gustation, colour, emotion-related) with the same lateralization characteristics as seen in the episodic memory between verbal and visual information (Mion, Patterson et al. 2010). Recent structural and functional neuroimaging data support a model in which the anterior temporal lobe serves as a hub linking all the modality-selective regions (Guo, Gorno-Tempini et al. 2013).

Procedural memory, also known as skill learning, refers to the ability to acquire cognitive and behavioural skills and algorithms that subsequently operate automatically with no individual awareness that the remembering occurred (IOM 2015). This type of memory is built up gradually over time as a function of practice (IOM 2015). Older adults do not usually have trouble doing procedures that are automatic or well learned. A good example of how procedural memory is preserved during ageing is the observation of elderly that find difficult to learn new information still maintain good procedural aspects on how to walk, dress or play an instrument (Midford and Kirsner 2005). The overall conclusion from research on implicit memory processes is that this aptitude stays relatively unimpaired in older adults, although greater age deficits may emerge when the implicit learning task becomes more complex (Martin and Li 2016). Evidence from functional neuroimaging and neuropsychological testing in patients with neurodegenerative diseases converge to implicate the basal ganglia, cerebellum and the supplementary motor area of the cortex as the brain regions critical to procedural memory (Grafton, Woods et al. 1994, Sabe, Jason et al. 1995).

The individuals cognitive reserve seems to explain the distinct performances found in aged population when comparing the working memory with other types of memory (Stern 2002). In accordance, within elderly individuals the degree of brain pathology does not correlate to their cognitive performance. It seems that certain features, like the extent of formal education, can be protective against age-related cognitive decline, which implies that in some individuals there are compensatory mechanisms that can prevent “expected” cognitive deterioration during senescence

(Stern 2009). This cognitive reserve is obviously more prominent when cognitive decline starts to occur during ageing and potentially explain part of the heterogeneity observed within intra-individual distinct cognitive domain performances and inter-individual ageing-cognition performance (Siedlecki, Stern et al. 2009). It is however still unclear how do the "protective" life exposures actually work and why are some cognitive dimensions more dependent of this cognitive reserve than others (de Mooij, Henson et al. 2018).

Detailed understanding of ageing and cognition depend on the precise characterization of individual's performance in various dimensions. For that purpose, several neurocognitive tests have been established and validated, as presented in table 2.

Table 1.2. Neuropsychological tests of memory. [Adapted from *Matthews et al.* in Continuum (Minneap Minn) 2015;21(3):613–626; (Matthews 2015)].

Memory type	Examples of Neuropsychological tests
Episodic (verbal)	Wechsler Memory Scale, 4th edition, Logical Memory: recall of oral narrative; California Verbal Learning Test, 2nd edition: list learning with 5 encoding trials;
Episodic (visual)	Brief Visuospatial Memory Test, revised: recall of simple figures scored for accuracy of shape and placement; Rey-Osterrieth Complex Figure Recall: immediate and delayed recall of copied complex figure;
Semantic (verbal)	Wechsler Adult Intelligence Scale, 4th edition, Information Test: fund of general knowledge; Boston Naming Test: naming line drawings;
Semantic (visual)	Northwestern University Famous Faces: recognition and identification of famous faces;
Working	Wechsler Adult Intelligence Scale, 4th edition, Digit Span. Wechsler Adult Intelligence Scale, 4th edition, Spatial Span.
Procedural	Not generally assessed with standardized tools.

Executive function

Executive function describes a wide range of cognitive aptitudes that relate to the capacity to adapt to a novel situation. This includes the ability to generate thought and think flexibly (*cognitive flexibility*, *set shifting*), to update and manipulate information mentally (*working memory* – see above), to inhibit what is irrelevant to current goals (*response inhibition*), and to plan and adjust decisions/behaviours as appropriate. *Fluency* represents the ability to exploit the production and retrieval of verbal or visual information during a specific time, while avoiding repeating responses (Martin and Li 2016). There are three common types of fluency tasks: category (semantic), letter (phonemic) and design. Verbal fluency has been commonly viewed as a component of executive function. However, several studies suggest that the cognitive mechanisms underlying efficient organization of verbal retrieval and recall in word generation are multidimensional and involve auditory attention, short-term memory, ability to initiate and maintain word production set, cognitive flexibility, response inhibition capacity, speeded mental processing and long-term vocabulary storage (Parkin and Lawrence 1994, Crowe 1998). Like many cognitive dimensions, it is difficult to assess pure executive function because many of the tests used to assess it also rely upon other cognitive processes, such as working memory, processing speed, attention and visual-spatial capacities. Generally speaking, executive function decays with ageing (Zelazo, Craik et al. 2004). When solving a problem that involves novelty or increased complexity (*e.g.*, requiring the ability to distinguish relevant from irrelevant information), older adults' performance tends to be worse than that of younger adults (Souchay and Isingrini 2004, Lesak, Howieson et al. 2012). Deficits in executive functioning like difficulties with planning and organizing, implementing strategies or even altered social behaviour can be seen during cognitive senescence.

Tasks requiring executive function activate neural networks that involve the prefrontal cortex, but also the parietal cortex, basal ganglia, thalamus and cerebellum (Collette, Van der Linden et al. 2005, Monchi, Petrides et al. 2006). The dorsolateral prefrontal cortex is involved during set shifting, planning and working memory (Stuss, Floden et al. 2001). The right prefrontal cortex is specialized in self-monitoring and spatial tasks, whereas left hemisphere regions are engaged in verbal processing (Baldo, Shimamura et al. 2001). When using working memory, the ventrolateral prefrontal cortex is active for recovery and conservation of information while the dorsolateral prefrontal regions are chosen for handling or updating of information (Wager and Smith 2003). The anterior cingulate cortex plays an important role in error detection (Devinsky, Morrell et al. 1995). Inferior frontal regions (*i.e.* ventrolateral prefrontal and orbitofrontal cortex) are critical for shifting

reward-punishment contingencies and for inhibiting inappropriate responses. Executive control is also supported by subcortical structures via their roles in cortico-basal ganglia and thalamocortical circuits and paralimbic areas (Schmahmann and Pandya 2008). Like the hippocampal system, neuron loss is not a prominent feature of ageing in many frontal lobe areas that mediate information processing functions known to decline with age. Instead, recent investigation has documented a substantial decline in the density of prefrontal cortex synapses, marked changes in dendritic architecture, and abnormalities in myelination and in white matter connections (Dumitriu, Hao et al. 2010, Morrison and Baxter 2012, Rabinovici, Stephens et al. 2015). Due to its neuro-anatomical extension and heterogeneity, executive functions are also vulnerable to white matter injury and to disturbances in the cholinergic, noradrenergic, serotonergic and dopaminergic neurotransmitter systems (Rabinovici, Stephens et al. 2015). Thus, various aspects of executive function can decline independently during ageing by some specific regional disturbance and not because of a global, brain-wide degenerative process.

Table 1.3. Neuropsychological tests of executive functions. [Adapted from *Rabinovici et al.* in Continuum (Minneap Minn) 2015;21(3):646–659;*(Rabinovici, Stephens et al. 2015)*].

Executive Function Domain	Examples of Neuropsychological tests
Working memory	Wechsler Adult Intelligence Scale, 4th edition, Digit Span Subtest: repeat a series of numbers, forward and backward; Corsi Block-Tapping Test or Wechsler Adult Intelligence Scale, 4th edition, Spatial Span Subtest: repeat a tapping sequence of up to nine blocks, forward and backward;
Inhibition	Stroop test such as the Colour-Word Interference Test from the Delis-Kaplan Executive Function System: name the ink colours of colour words that are printed in discordant ink; Flanker test or Continuous Performance Test from the National Institutes of Health Executive Abilities, Measures of Instruments for Neurobehavioral Evaluation and Research: indicate the direction the centre arrow is pointing as quickly as possible, while ignoring flanking arrows that, on some trials, point in the opposite direction (Flanker test); or respond to the target image and withhold responses to distractor images (Continuous Performance Test);

Set shifthing	Trail Making Test from the Delis-Kaplan Executive Function System: draw lines that connect numbers and letters in alternating and ascending order; Wisconsin Card Sorting Test: sort cards according to changing rules and based on examiner feedback;

Language function

The *language function* refers to an array of abilities, including understanding and producing speech, naming, reading and writing (Verhaeghen 2013). It is a primary instrument for human communication and a critical element of many other cognitive tasks and social interactions. Two main areas of verbal abilities are frequently discussed: *verbal fluency* (semantic and phonemic – see above) and *confrontation naming* (the ability to identify an object by its name). Most verbal abilities remain intact in older adults (Martin and Li 2016). Therefore, vocabulary and verbal testing scores remain relatively stable during ageing and may even show some minor improvements (Zec, Markwell et al. 2005, Martin and Li 2016). Despite this preserved vocabulary and comprehension, language production skills do decline with age (Martin and Li 2016). These include word-finding failures and language disfluencies (*e.g.*, "having a word on tip of the tongue") (Rapp and Bachevalier 2012). The hearing loss observed in some elderly may reduce further the understanding of spoken language (Wingfield, Tun et al. 1999, Mick, Foley et al. 2014). It is commonly agreed that language is highly dependent on structures within the left peri-sylvian region of the brain (Rapp and Bachevalier 2012). Neuroimaging and lesion studies provide corroboration to the empirically derived theories that point to an interplay of various neurocognitive systems for the language function. For example, current studies in verbal fluency (word generation and retrieval) associate the following brain structures: left dorsolateral prefrontal cortex, inferior frontal cortex (Broca's area), anterior cingulate gyrus, both left and right frontal lobes, basal ganglia, hippocampal formation and posterior para-hippocampal gyrus, as well as the right cerebellum (Audenaert, Brans et al. 2000, Fama, Sullivan et al. 2000, Leggio, Silveri et al. 2000, Crosson, Benefield et al. 2003). The considerable heterogeneity seen in patterns of language decline during ageing remains poorly understood, suggesting that this extensive cognitive system is possibly subject to multiple influences that modulate damage and cognitive reserve.

Attention

Attention is the capacity for processing information and relates to the ability to focus and concentrate on a given stimuli for a sustained period of time (Verhaeghen 2013). One can only process mentally a limited amount of data at the same time. Attention allows the brain to function effectively by selecting the specific information to be analysed and processed at the same time, filtering out all the unnecessary data presented to the sensory nervous system. Models of attention classically divide attention into various processes, such as alertness (arousal), selective, divided and sustained attention. *Alertness* is defined by the creation of a state of arousal or readiness by an unexpected external cue. *Selective attention* refers to the ability to filter data and focus on specific items despite the presence of other information. *Divided attention* is referred to the ability to split the focus between competing activities or multiple sources of information (multitasking) and can involve the processing of multiple information's or tasks performances simultaneously. *Sustained attention* refers to the aptitude of maintaining concentration on a task for a long period. Attentional processes are particularly vulnerable to the process of ageing (Martin and Li 2016). In general, attention on simple tasks is relatively well preserved into the 80s but as the complexity increases or divided attention is required, older adults tend to respond more slowly and with increased number of errors (Zanto and Gazzaley 2014). The elderly also find more difficult to perform multitasking actions and to discriminate between relevant and irrelevant stimuli, like locating relevant information in the presence of distracting background information (Verhaeghen and Cerella 2002). The literature suggests that sustained attention does not usually show any age-related deterioration (Berardi, Parasuraman et al. 2001).

All these three attention processes interact with sensorimotor processing systems, operate across distinct sensory modalities and can be modulated by motor input (Rizzolatti, Riggio et al. 1987, Matusz, Broadbent et al. 2015). For example, in visual attention, brain functional imaging data obtained during such cueing tasks have revealed that networks supporting each of these attention processes were vast but mostly independent. A*lertness* was linked to the *locus coeruleus*, right parietal and frontal; *selective* and *divided attention* with the activation of the frontal eye fields, superior parietal junction, superior temporal junction, superior colliculus and pulvinar as well as the anterior cingulate, anterior insula, frontal cortex and striatum (Fan, McCandliss et al. 2002, Amso and Scerif 2015).

Decline in cognitive function with age has sometimes been viewed as an inevitable outcome of the ageing process. However, today, we recognise that cognitive features and trajectories throughout senescence are not homogeneous within or between individuals. Unfortunately, it seems to depend on factors whose interactions remain mostly unknown (Albert, Jones et al. 1995, Pilling, Harries et al. 2012).

Some of the many relations between Ageing and Cognition may be modulated by the Endocrine system, which will be discussed next.

1.4 Some Endocrine perspectives on Ageing and Cognition

The current definition of the endocrine system covers the integrated network of multiple organs that release substances (from steroids to small peptides and glycoproteins) that will have their effect in distant or close target cells – hormones (Molina 2018). The primordial function of the endocrine system is to coordinate and integrate cellular and organ activity within the whole body, ensuring and maintaining a homeostatic internal environment throughout the lifespan. This complex system interacts intensively with the central and peripheral nervous systems leading to the present notion of a "neuro-endocrine" system.

The fact that hormones have a fundamental role in so many aspects of organism's functioning has led, very early in the human science history, to the idea that hormones were the key to ageing. In the XIX century the neurologist *Charles Brown-Séquard* anticipated that testicular extracts from guinea pigs or dogs could restore vitality and provide a mean to revert some ageing features leading him to regularly self-inject a homemade *elixir* (Brown-Sequard 1889). Today it is recognized that sex-steroids hormones coming from the gonadal axis are molecules with positive effects on some age-related features such as sarcopenia, bone-mass, cardio-vascular and libido/sexual dysfunction, dementia and even mortality (Barron and Pike 2012, Ahern and Wu 2015, Davis, Lambrinoudaki et al. 2015). In the 1960's, *Everitt* and *Cavanagh* demonstrated that the removal of the pituitary gland in rats induced a delay of the ageing process in tail tendon collagen fibres and in the onset of proteinuria (Olsen and Everitt 1965). Considering these observations, they postulated that some kind of anti-ageing and/or life-maintaining factor was secreted by the pituitary gland. Several hormones are produced and secreted by the pituitary, some of which currently known to affect directly the biological mechanisms linked to ageing. One of the most relevant hormonal modulator of cellular and organism senescence is the GH-IGF1 (somatotropic) axis and its connection to insulin sensitivity (Tatar, Bartke et al. 2003).

As referred before, ageing has implications on multiple organs and systems. The endocrine system is no exclusion, but the effect of time on this integrative network varies substantially depending on the hormone axis studied and on individual's features. Most hormone axes present only a modest decline, while others, like the gonadal, undergo profound changes during the lifespan. In women, dramatic and abrupt drop in the ovarian estrogenic production starts around the 5th decade of life. The cycling fluctuation of oestradiol during the reproductive years is then replaced by a low,

constant oestradiol levels with exhaustion of the ovary follicles and reproductive senescence – menopause (Davis, Lambrinoudaki et al. 2015). A more stable decline loss of sexual steroids occurs in men. Unlike women, there is no specific age at which this process starts. Its beginning is attributed to the downregulation of hypothalamic-pituitary-testicle axis which will eventually lead to testosterone deficiency (Araujo and Wittert 2011). In general, during ageing, total energy expenditure decreases, primarily due to a decline in physical activity. An age-associated reduction in the resting metabolic rate may actually reflect more a loss of fat-free mass and not just a simple direct effect of any particular hormonal modification (Masoro 2016). Impaired glucose tolerance is also seen during ageing. This may result from a small decrease in insulin secretion and an increase in insulin resistance, as a consequence of simultaneous body adiposity percentage increase and physical activity decline (Masoro 2016).

Given the broadness and extension of the subject, and the nature of this thesis, I will specifically focus on the following endocrine axes and pathways: vitamin D, hypothalamic-pituitary-adrenal, hypothalamic-pituitary-thyroid and somatotropic axes. Each will be discussed in more detail in the next sections, exploring the current knowledge about their interactions with ageing and cognition.

1.5 Vitamin D

Vitamin D is a secosteroid first described as present in cod liver oil and used, in the early XX century, to effectively treat rickets (DeLuca 1988). It is a unique nutrient because its supplies are met mainly through skin exposure to sunlight (~80%) and only slightly obtained from the diet (Papadimitriou 2017). In mammals, vitamin D derived from these two sources is not biologically active; it requires further processing. Cholecalciferol (vitamin D3) is produced by the skin, from 7-dehydrocholesterol (7HC), when exposed to ultraviolet B radiation (wavelength 280-315 nm) but also by ingesting animal products enriched in vitamin D3 (Molina 2018). Another source of vitamin D is that obtained by laboratory chemically alteration of ergocalciferol (vitamin D2) obtained from plant or fungi. Both vitamin D2 or D3, bound to vitamin D-binding protein (Schuster 2011, Jones, Scheuerlein et al. 2014), are carried in the bloodstream to the liver where they undergo the first step of bioactivation (hydroxylation), by CYP2R1, into 25-hydroxyvitamin D [25(OH)D, calcidiol], the major circulating form of vitamin D. To achieve the final active form, an additional hydroxylation takes place by the CYP27B1 (1α-hydroxylase), mainly in the kidney, with the formation of $1,25(OH)_2D$ (calcitriol) (Schuster 2011). The 1α-hydroxylase function is tightly controlled via

feedback mechanisms mainly from the parathyroid hormone (PTH) and plasma calcium, but also from phosphate, calcitonin, fibroblast growth factor 23 (FGF23) and $1,25(OH)_2D$ itself (Lehmann and Meurer 2010). High levels of plasma calcium will suppress the active hydroxylation of 25(OH)D. By the contrary, a rise in PTH will stimulate the activity of 1α-hydroxylase increasing the synthesis of calcitriol. Even though 1α-hydroxylase is mainly expressed within the kidney, a variety of other tissues also present this enzyme, including the skin, immune cells and pancreas (Zehnder, Bland et al. 2001). This extra-renal presence suggests an autocrine/paracrine mechanism and may explain some of the kidney independent effects of vitamin D (Adams and Hewison 2012).

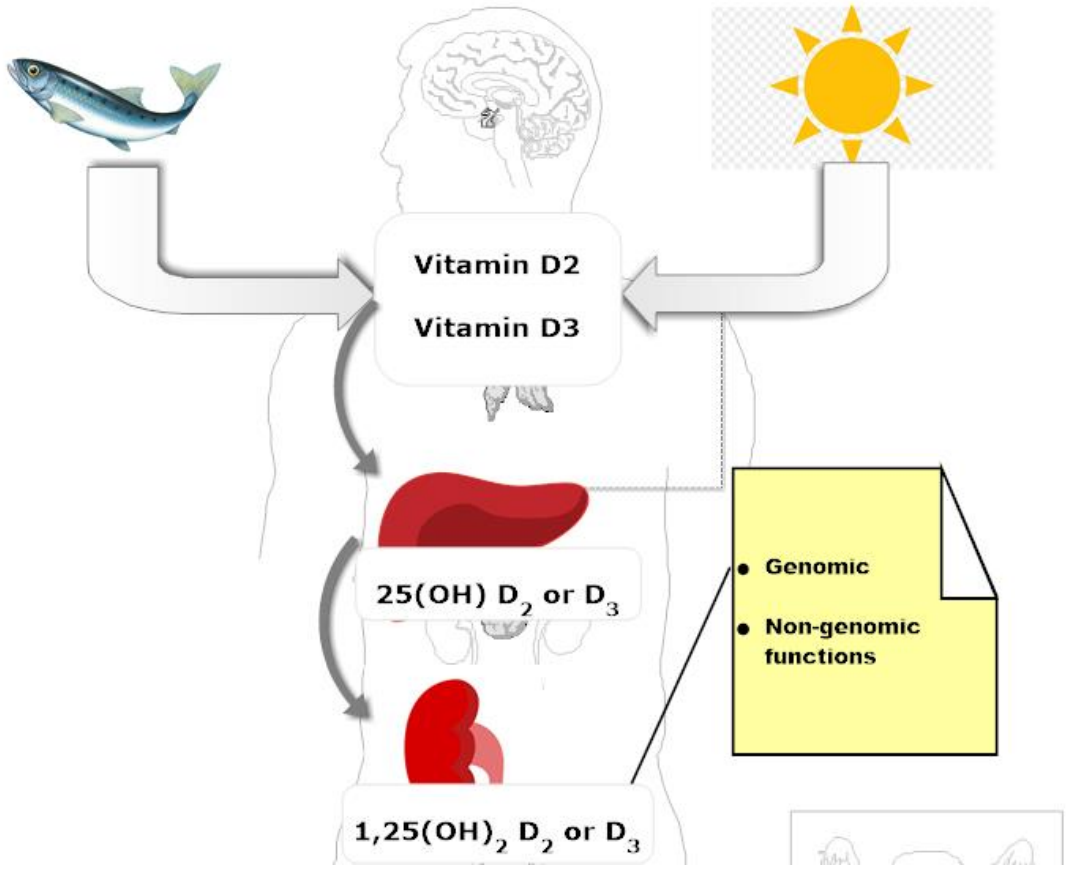

Figure 1.9. Overview of vitamin D activation and mechanism of action in humans. Adapted from *Jamali, Sorenson et al. 2018* (Jamali, Sorenson et al. 2018).

Calcitriol produces a wide spectrum of biological responses through interaction with both its nuclear and cellular membrane receptors. The genomic actions result from $1,25(OH)_2D$ binding to the nuclear vitamin D receptor (VDR) (Haussler, Whitfield et al. 2013). This VDR is a member of the

steroid/thyroid superfamily of nuclear transcription factors and is present in almost all body nucleated cells (Bouillon, Carmeliet et al. 2008, Wang, Zhu et al. 2012). After binding, calcitriol generates conformational changes of VDR followed by heterodimerization with unliganded retinoid X receptor, recruiting vitamin D response elements and releasing of corepressors with activation of the transcriptional complex (Haussler, Whitfield et al. 2013). Non-genomic rapid response to $1,25(OH)_2D$ is started through either a membrane-bound VDR or a protein-disulfide isomerase-associated 3 protein (Haussler, Jurutka et al. 2011). The activation of second messenger signalling pathways (phosphoinositide turnover, activation of protein kinase C and the Ras-Raf-ERK-MAPK pathway) will then alter the phosphorylation states of cellular proteins and start a cascade of various cellular processes (Haussler, Jurutka et al. 2011).

The classic target tissues for calcitriol are the ones involved in the calcium and phosphate metabolism – bone, intestine, kidney and parathyroid glands. Non-calcemic or non-classic actions for vitamin D are also well recognized particularly after the acknowledgement that a very large number of genes are under direct or indirect control of $1,25(OH)_2D$ (Ramagopalan, Heger et al. 2010). This widespread action is in line with other ligands for nuclear receptors, such as glucocorticoids, androgens, oestrogens, thyroid hormones and retinoids, which also present an extensive spectrum of activities (Bouillon, Carmeliet et al. 2008). Several studies in animal models and in humans lead to the current idea that the functioning of nearly all major tissues or systems of the organism is somehow modulated by vitamin D (Overbergh, Decallonne et al. 2000, Rosen 2011, Michos, Carson et al. 2014, Gil, Plaza-Diaz et al. 2018).

Defining vitamin D status

Plasma or serum 25(OH)D concentration is commonly used as a surrogate biomarker of vitamin D status. It reflects vitamin D supply from cutaneous synthesis and from diet and presents a relatively long half-life in the circulation (about 2-3 weeks) (Bouillon 2016). Some limitations of its use are the decrease in its plasma concentration in response to acute inflammation (consequently, low plasma levels may result from an underlying inflammatory state) and the confounding influence of seasonal and cultural distinct sun exposures as well as some geographical/genetic variation (Bouillon 2016).

Currently, there is no consensus over what should be the optimal 25(OH)D plasma levels (Giustina, Adler et al. 2019). Based only on calcium and bone metabolism studies, and some observational

data coming from the healthy adult population, the American National Academy of Medicine (formerly called the Institute of Medicine) issued, in 2010, a report saying that vitamin supplementation was unlikely to be beneficial for individuals with 25(OH)D blood concentrations of 50 nmol/l (20 ng/ml) or greater (Ross, Manson et al. 2011). Several other organizations and authors have a different opinion. Based on data originating from non-calcium dependent health outcomes, the Endocrine Society and others have recommended that 25(OH)D levels higher than 75 nmol/l (30 ng/ml), with the preferred range of 100–150 nmol/l (40–60 ng/ml), are necessary to avoid vitamin D deficiency (Holick, Binkley et al. 2011).

Table 1.4. Diverse recommendations for interpreting vitamin D status based on total serum levels of 25(OH)D.

	Below 25-30 nmol/l	30-50 nmol/l	50-75 nmol/l	75-100 nmol/l	Above 100 nmol/l
	Below 10-12 ng/ml	12-20 ng/ml	20-30 ng/ml	30-40 ng/ml	Above 40 ng/ml
NAM (IOM), AAP, DGS, Nordic and many Central European Countries, Australia	Severe Deficiency	Deficiency	Sufficiency	Sufficiency	Sufficiency
Endocrine Society, IOF, AGS	Severe Deficiency	Deficiency	Mild Deficiency	Sufficiency	Sufficiency
Vitamin D Council	Severe Deficiency	Deficiency	Mild Deficiency	Mild Deficiency	Sufficiency

AAP, American Academy of Paediatricians; AGS, American Geriatric Society; DGS, *Direcção-Geral da Saúde* (Portuguese Director General of Health); IOF, International Osteoporosis Foundation; IOM, Institute of Medicine; NAM, National Academy of Medicine. Adapted from *Bouillon, R. Nature Reviews Endocrinology,* (Bouillon 2017) and *Technical guidance n13, 2008, from the Portuguese Director General of Health* (Direcção-Geral da Saúde 2008).

In an extensive meta-analysis of cross-sectional studies on vitamin D status in healthy subjects around the world (394 studies), the mean serum 25(OH)D levels was of 54 nmol/l (Hagenau, Vest et al. 2009). Remarkably, latitude didn't have a major influence, demonstrating perhaps that apart

from potential exposure to UVB light, other factors (skin pigmentation, lifestyle and nutritional factors) are potentially more decisive to the vitamin D status. In a more recent review, the mean population- 25(OH)D values were very different between countries (range 4.9–136.2 nmol/l), with almost 40% of the studies reporting mean values below 50 nmol/l, with no significant age- or sex-related differences (Hilger, Friedel et al. 2014). The highest 25(OH)D values were observed in North America, possibly reflecting a more implemented diet supplementation policy. To date, in Portugal only a few population-based studies have provided data on vitamin D status in adults. In a nationwide cluster sample of 1,500 Portuguese subjects over ≥65 years of age, *Santos et al.* found a median 25(OH)D serum value of 36.1 nmol/l with 69% of the participants under the 50 nmol/l threshold (Santos, Amaral et al. 2017). In another cross-sectional study evaluating a sub-sample population of 500 Portuguese with a median age of 53 years, the median 25(OH)D level obtained was 34.5 nmol/l with 86% of the participants below the proposed level of adequacy (Raposo, Martins et al. 2017). More recently in a somewhat younger population from the north of Portugal (198 individuals, age range between 18 and 67 years) the mean level of serum 25(OH)D was 55.4 nmol/l, with 48% presenting values below 50 nmol/l (Bettencourt, Boleixa et al. 2018). These results are in line with data coming from neighbouring southern European countries. In a population-based cohort study in Spain with more 2,200 participants, vitamin deficiency was reported in 33.9% (below 50 nmol/l) with a median 25(OH)D levels of 56.2 nmol/l (Gonzalez-Molero, Morcillo et al. 2011). In a prospective population-based study, performed in a rural area of Italy, *Houston et al.* found a similar mean serum 25(OH)D concentration of almost 58 nmol/l with significant lower levels in men and in older individuals, and about 51% of vitamin deficiency (below 50 nmol/l) prevalence (Houston, Cesari et al. 2007).

Importantly there are also some limitations associated with the methods used for 25(OH)D measurements. Multiple methodologies for total 25(OH)D determination [$25(OH)D_2$ and $25(OH)D_3$] exist, including immunological, high-pressure liquid chromatography and liquid chromatography tandem mass spectroscopy (Holick 2009). Variations between measurements can fluctuate considerably (15-20%) depending on the type of assay used and inclusion/exclusion of $25(OH)D_2$ value (Cashman, Dowling et al. 2016). In addition, there are also considerable differences between laboratories using the same methods (Binkley, Dawson-Hughes et al. 2017). These multiple limitations have raised considerable concerns into defining optimal 25(OH)D cut points and how to interpret correctly the various studies evaluating serum 25(OH)D levels with distinct health outcomes. These discrepancies are likely to have deleterious consequences of public health

concern, since the lay literature is reporting on severe vitamin D in unlikely settings, which increases the drive to demand and prescribe vitamin D supplements (Pilz, Zittermann et al. 2019).

Vitamin D and ageing

In adults, vitamin D deficiency can lead to osteomalacia, presenting as muscle weakness and bone tenderness or pain (Bouillon 2016). Vast evidence suggests that low levels of 25(OH)D can also increase the risk of many other acute and chronic diseases (*e.g.* infections, cancer, cardiovascular and auto-immune diseases), as well as all-cause mortality (Pludowski, Holick et al. 2013, Schottker, Jorde et al. 2014). Cross-sectional studies in the 1980's proposed that older adults present lower serum 25(OH)D than younger people and therefore could be at higher risk for these disorders (Baker, Peacock et al. 1980). There was a rationale for this age-dependent link. Old people tend to spend less time outdoors, wear longer clothes and produce less 25(OH)D due to the decrease of the skin vitamin D precursor (Lester, Skinner et al. 1977, MacLaughlin and Holick 1985). However, more recent studies evaluating older people living independently did not find these 25(OH)D differences when compared to younger dwellers (Hilger, Friedel et al. 2014). It is still not known whether the decreased kidney function observed in ageing has any relevant effect on the overall individual's vitamin D status. Despite the relative low importance of dietary vitamin D on the total 25(OH)D levels, available data points to a lower intake of vitamin D in older individuals and increased susceptibility to vitamin D inadequacy due to a poorer diet (Quann, Fulgoni et al. 2015, Castiglione, Platania et al. 2018).

There is an increasing evidence that vitamin D may have, itself, a role in modulating cellular senescence. The basis for this hypothesis comes from the awareness that several of the processes that determine cellular ageing are regulated by vitamin D (Berridge 2017). In some cellular models, calcitriol potentiates autophagy – one of the main hallmarks of ageing (Jang, Kim et al. 2014). In others, it has been demonstrated that inflammation, and the *inflammageing* phenotype associated with organism senescence, was reduced by 25(OH)D administration probably by moderating the expression of inflammatory cytokines (Beilfuss, Berg et al. 2012, Alvarez, Chowdhury et al. 2014). Reinforcing this idea many other studies have also shown that mitochondrial dysfunction (with deregulated reactive oxygen species production), telomere trimming and some epigenetic profiling associated to ageing are potentiated in vitamin D deficiency models (Richards, Valdes et al. 2007, Pereira, Barbachano et al. 2012, Consiglio, Viano et al. 2015, Ryan, Craig et al. 2016). Vitamin D

also seems to modulate vascular tone by stimulating nitric oxide production, through activation of nitric oxide synthase, and by regulating expression of many endothelium-derived contracting factors (*e.g.* arachidonic acid and cyclooxygenase-1) (Bukoski, DeWan et al. 1989, Molinari, Uberti et al. 2011). Loss of the normal vascular tone response and the increase of arterial stiffness are two well-known features associated with ageing (Akhtar 2017). The precise role of vitamin D deficiency in premature ageing of the cardiovascular system is still a matter of debate (Hiemstra, Lim et al. 2019).

Vitamin D and cognition

The presence of VDR and 1α-hydroxylase in the brain was first recognized more than a decade ago but its actual distribution is still controversial (Prufer, Veenstra et al. 1999). In humans, the presence of VDR in the cortex, hippocampus and brain nucleus was proposed by many authors but not all agree with this widespread distribution (Eyles, Smith et al. 2005, Wang, Zhu et al. 2012). Some of these contradictory findings may result from important variations found when using different staining methods.

Animal studies

In *animal studies*, vitamin D was shown to reduce inflammation-induced degeneration of neurons but also to increase amyloid clearance – in support of impaired vitamin D status in neurodegenerative diseases of the central system (Lefebvre d'Hellencourt, Montero-Menei et al. 2003, Briones and Darwish 2012). Structural cortex alterations and behavioural modifications have also been observed in rats exposed to a vitamin D deficient environment during early life (Feron, Burne et al. 2005, Turner, Young et al. 2013). In rats born from vitamin D deficient mothers, profound brain alterations were found at birth (increased total brain volume and cell proliferation indexes but proportionally thinner cortex) (Eyles, Brown et al. 2003). However, few studies have examined the prolonged effect of vitamin D deficiency on behaviour (Byrne, Voogt et al. 2013, Groves, Kesby et al. 2013). According to the present literature, the absence of vitamin D during growth seems to be crucial for the orderly cascade of brain development whereas, in contrast, low vitamin D during adulthood has only been associated to some subtle changes in brain neurochemistry and behaviour (Kesby, Cui et al. 2010). This heterogeneity does not provide a clear answer about the role of vitamin D deficiency in brain function in ageing rodents.

Human studies – brain morphology and function

In humans, only a few *cross-sectional studies* have evaluated potential brain morphometric changes associated to vitamin D status. In a systematic review with *meta-analysis* of all the work published until 2014, *Annweiler et al.* found that vitamin D depletion was associated with smaller brain volume and enlarged lateral ventricles (Annweiler, Annweiler et al. 2014). Brain sub-volumes data were mixed and insufficient to conduct a full meta-analysis. In a study involving 92 healthy persons, mean age 63 years, the authors showed that a higher rate of brain atrophy of the prefrontal cortex was associated to subjects with lower 25(OH)D plasma concentrations (Hooshmand, Lokk et al. 2014). Until now, no connection between vitamin D and cortical grey matter volume was presented in any of the studies addressing this question in healthy individuals (Annweiler, Annweiler et al. 2014). The only published work reporting a possible link between current 25(OH)D levels and grey matter volume was a case-control study evaluating Norwegian patients with psychosis *versus* healthy controls. In this report *Berg et al.* found that, in the patients group, 25(OH)D serum concentrations had a positive correlation with peripheral grey matter, white matter and whole brain volumes (Berg, Jorgensen et al. 2018). More interestingly, a recent study evaluating 110 older community-dwellers (mean age 72 years), demonstrated a negative correlation between 25(OH)D levels and intracranial and white matter volumes, even after multiple adjustments. No other volumes evaluated showed association to the vitamin D status of the individuals (Annweiler, Bartha et al. 2015). There are several potential explanations for some of these unexpected results. Cranial volume is highly dependent on hereditary traits and on early life nutritional status. Higher levels of 25(OH)D were linked to earlier closure of cranial fontanelles, lower head circumference and consequent decreased total brain volume (Kumar, Sachdev et al. 2011). Since observational studies do not allow to infer causality, it remains to be clarified whether these early effects of vitamin D, or even some kind of population selection bias, were responsible for part of the results assessing the 25(OH)D effect on human brain morphometric features.

Human studies – neuro-cognitive performance

Most *observational studies* support an association between low levels of 25(OH)D and minor cognitive performance in old adults. Several published *cross-sectional studies* showed that older adults with low 25(OH)D status perform cognitively worse than those with higher levels. Most of these studies provided only data on global screening tools like MMSE [see review by *Balion et. al.* (Balion, Griffith et al. 2012)]. The few with separated analysis for each neuropsychologic test and cognitive dimension have presented contradictory results, sometimes even within the same report (Balion, Griffith et al. 2012, Granic, Hill et al. 2015, Lam, Albrecht et al. 2016, Pettersen 2016).

The current published *prospective studies* do not help to clarify these issues. Some described that specific cognitive domains seem to be more vulnerable to 25(OH)D deficiency. For instance, data coming from the Third National Health and Nutrition Examination Survey (NHANES III) demonstrated that cognitive impairment in the elderly population of USA, specifically verbal memory, orientation and attention, was associated with vitamin D deficiency (Llewellyn, Lang et al. 2011). In a large study with 6,257 older women followed for 4 years, low baseline 25(OH)D serum concentrations were also associated with overall cognitive decline but not with executive performance (Slinin, Paudel et al. 2012). In another population-based study, a "deseasonalized" 25(OH)D concentration was positively correlated with semantic memory scores and, more recently, a longitudinal study with an extended 10-year follow-up of 252 women showed that, in midlife, vitamin D above 25nmol/l was associated with improved aspects of executive function later in life (Annweiler, Milea et al. 2016, Goodwill, Campbell et al. 2018). By contrary, a large prospective study in osteoporotic male found no link between lower 25(OH)D levels and baseline global and executive cognitive function with little evidence for its association with incident cognitive decline during a mean follow-up of 4.6 years (Slinin, Paudel et al. 2010). It is unknown whether these differences are related to gender. Additional longitudinal studies have shown that some cognitive domains, including memory and attention, were not linked to baseline vitamin D status (Buell, Scott et al. 2009, Llewellyn, Lang et al. 2010). In a mixed prospective report including 1,184 women, higher levels of plasma vitamin D were associated with better cognitive function one decade later, but no association was seen with cognitive decline after the extended 6 years follow-up (Bartali, Devore et al. 2014). Similarly, in a large USA-based cohort spanning over 20-years, 25(OH)D levels measured in midlife were not associated to increased cognitive decline over the follow-up period (Schneider, Zhao et al. 2018). These inconclusive observational findings resulted in contradictory assumptions about the link between 25(OH)D levels and memory, executive function, general cognitive tests, as well as with neurocognitive decline during ageing. Many of the "positive" results may have occasioned by reverse causality and by some methodological defects contributing to the excessive heterogeneities observed: single point 25(OH)D evaluations, population selection bias, single age-group, distinct vitamin D statuses at baseline or neuropsychological assessment (see below).

Table 1.5. Key points about vitamin D and brain function.

• Vitamin D is linked to reduction of inflammation-induced neurodegeneration in animal models
• Low levels of Vitamin D are associated to reduced human adult brain volume (PFC more susceptible?)
• The most consistent human neuro-cognitive domain modified by 25(OH)D levels is Visual Memory
• Reverse causality and excessive heterogeneity among human studies impair definite conclusions

Interventional studies have also provided limited clarification. To date, eight studies have been published assessing the effect of vitamin D supplementation on cognitive performance in adults (Dhesi, Jackson et al. 2004, Przybelski, Agrawal et al. 2008, Dean, Bellgrove et al. 2011, Stein, Scherer et al. 2011, Rossom, Espeland et al. 2012, Pettersen 2017, SanMartin, Henriquez et al. 2018, Castle, Fiedler et al. 2019). All of them have significant design weaknesses. *Rossom et al.* performed a *post hoc* analysis of cognition-related outcomes of 4,143 women enrolled in the Women's Health Initiative calcium and vitamin D trial and the Memory Study where they were randomised to receive cholecalciferol and calcium carbonate or placebo. During the follow-up (mean 7.8 years), vitamin D supplementation (with calcium) was found to have no effect on cognitive decline or incident dementia (Rossom, Espeland et al. 2012). Dean *et al.* studied vitamin D supplementation in 128 young adults for 6 weeks and found no influence on working memory, response inhibition or cognitive flexibility (Dean, Bellgrove et al. 2011). More recently, 82 healthy adults from Canada were randomized to high (4,000 IU/day) or low dose (400 IU/day) of cholecalciferol for 18 weeks (Pettersen 2017). A positive result for higher doses was observed on visual memory, particularly among those who presented 25(OH)D below 75 nmol/l at baseline, while verbal memory and other cognitive domains did not show differences. In a randomized controlled trial of vitamin D supplementation including only healthy postmenopausal women published in 2019, *Castle et al.* found that when exposed for one year to three distinct vitamin D3 daily supplements (600, 2,000, or 4,000 IU/d), only the intermediate dose group (2,000 IU) exhibited positive effects on visual and working memory and learning (Castle, Fiedler et al. 2019). The other four studies published were very small and examined the effects of vitamin D supplements on cognition in adults already with mild/moderate dementia or very high risk of fracture (Dhesi, Jackson et al. 2004, Przybelski, Agrawal et al. 2008, Stein, Scherer et al. 2011, SanMartin, Henriquez et al. 2018). No effect on cognitive decline or incident dementia was detected in all, but one of them.

To help summarize part of this evidence, a recent *meta-analysis* was published including twenty-six observational and three intervention studies (between 19 and 9,556 participants) (Goodwill and Szoeke 2017). It concluded that low vitamin D was associated with worse cognitive performance (OR = 1.24, CI = 1.14–1.35) and cognitive decline (OR = 1.26, CI = 1.09–1.23). The *cross-sectional studies* yielded a more robust association when compared to *longitudinal studies*. but the *interventional studies* with vitamin D supplementation showed no significant benefit on cognition (SMD = 0.21, CI = 0.05 to 0.46) (Goodwill and Szoeke 2017). The authors also concluded for a potential reverse causality bias linked to these observational results. Individuals with better cognitive performances may have a more active life, which could lead them to more outdoor and sun exposure in addition to healthier dietary intake of vitamin D. This, in turn, results in better serum 25(OH)D levels. Other relevant issue is the time needed for any variable to modulate cognition and potentially protect from dementia. Usually, it is thought to be measurable in decades, yet most of the available longitudinal evidence comes from studies with less than 5 years of follow-up, primarily done in elderly population and with only one time 25(OH)D determination. Another important issue is the variance in genetic polymorphisms of VDR and vitamin D binding protein (VDBP) between populations. These potential genetic differences have been proposed to justify why some individuals are more resilient to vitamin D deficiency and present adequate biologically response even at low 25(OH)D levels (Kuningas, Mooijaart et al. 2009, Powe, Evans et al. 2013). Currently, none of the published intervention trials provided a conclusive answer to the question of vitamin D effect on cognition. As such, lifespan cohort studies, addressing duration and optimal beneficial windows, with several separate 25(OH)D determinations based on good standard laboratory methods and, if possible, evaluating the existence of protective VDR/VDBP gene polymorphisms, are needed to better understand the role of this vitamin on cognition and healthy ageing.

Despite its weak influence on the overall 25(OH)D levels, dietary intake of vitamin D has also been linked to several cognitive outcomes. In a large French cross-sectional study involving 5,596 community-dwelling women (mean age 80 years), higher weekly median vitamin D intakes (above 35 mcg per week) were related to better global cognitive function (Annweiler, Schott et al. 2010). More recently, in a healthy ageing longitudinal study in the USA including 2,574 participants (overall mean vitamin D intake of 3.9 mcg per day), higher dietary intake of vitamin D was associated to slower rate of decline in the domain of verbal fluency in older women and black adults, and slower

rates of decline in verbal memory and in visual memory/visuo-constructive abilities were found among younger women and white participants, respectively (Beydoun, Hossain et al. 2018). Despite the lack of data reporting on serum 25(OH)D levels in these participants, some relevant evidence can be recognised mostly because dietary intake estimation can be positively correlated to 25(OH)D levels; be less dependent of the season and help to discriminate between endogenous and exogenous vitamin D origin and their link with the outcomes.

Raising more questions, some published data showed a positive effect of sun exposure over brain structure and cognitive performance, independently from the vitamin D intake or plasma levels (Zivadinov, Treu et al. 2013, Kent, Kabagambe et al. 2014). In a large observational community-based cohort study of more than 3,000 older persons, an average composite global cognitive function was higher during summer and fall compared to winter and spring, with the difference equivalent in cognitive effect to almost 5 years' difference in age, with the odds of meeting criteria for mild cognitive impairment or dementia being also lower (~30%) during summer and fall. These results persisted even after adjustment to multiple potential confounders like depressive symptoms, sleep, physical activity and thyroid status. No information was provided on 25(OH)D levels (Lim, Gaiteri et al. 2018). A molecular and cellular mechanism for this UV-induced neurobehavioral changes has been recently proposed in mice and involves the increased synaptic release of glutamate which potentiated motor learning and memory (Zhu, Wang et al. 2018). Based on these results it is reasonable to expect that cognitive outcomes associated with plasma 25(OH)D levels could be partially explained by the sun-light exposure, placing the vitamin D status as a surrogate marker of increased outdoor presence and UVB light exposure.

Table 1.6. Relation between 25(OH)D levels and distinctive neurocognitive features.

Neurocognitive feature		25(OH)D relationship	Reference examples
Global cognition		POSITIVE	Balion, Griffith et al. 2012
		NEUTRAL	Slinin, Paudel et al. 2012;
Cognition decline during ageing			
		INVERSE	Slinin, Paudel et al. 2012;
		NEUTRAL	Bartali, Devore et al. 2014;
Executive function			
	Working memory	POSITIVE	Annweiler, et al. 2013;
		NEUTRAL	Slinin, Paudel et al. 2010;
	Set shifting	POSITIVE	Goodwill, Campbell et al. 2018;
Memory			
	Semantic	POSITIVE	Annweiler, Milea et al. 2016;
	Verbal	POSITIVE	Llewellyn, Lang et al. 2011;
		NEUTRAL	Goodwill, Campbell et al. 2018;
	Visual	POSITIVE	Petterson et al. 2017
Attention		POSITIVE	Llewellyn, Lang et al. 2011;

1.6 Hypothalamus-pituitary-adrenal axis

In mammalian vertebrates, the hypothalamus-pituitary-adrenal (HPA) axis is a vital adaptative (allostatic) system. It comprises primarily the hippocampus and frontal cortex; catecholaminergic tracts; corticotropin-releasing hormone (CRH) and antidiuretic hormone arginine vasopressin (AVP) produced by the medial parvocellular region of the hypothalamic paraventricular nucleus (PVN); adreno-corticotropic hormone (ACTH) produced by pituitary corticotropes, and cortisol secreted from the zona *fasciculata* of adrenal cortex (Veldhuis 2013). Autoinhibition and a negative feedback loop helps to regulate this glucocorticoid secretion. CRH neurons in the medial parvocellular part of the PVN respond to hypothalamic CA1 neurons and to other afferences, releasing CRH from their neurosecretory nerve terminals (at the median eminence) into the primary plexus of the pituitary portal circulation (David Norris and Carr 2013). In the anterior pituitary lobe, corticotropes respond to CRH by secreting ACTH. The presence of AVP, arising from the magnocellular elements of the PVN neurosecretory pathways, increase corticotropes sensitivity to CRH and further intensify their ACTH response (Molina 2018). From the pituitary, ACTH will follow systemic circulation, to the adrenal cortex where it will finally stimulate cortisol secretion. Cortisol elevation in the blood feeds back to inhibit both the hypothalamus part of the circuit and the pituitary corticotropes, therefore closing the retro-controlled loop of the HPA axis.

The activity of the HPA axis and glucocorticoid secretion divides into 3 distinct patterns: basal ultradian pulses, basal circadian fluctuation and stressor-induced activity. Most mammals, including humans, have an ultradian rhythm of basal ACTH and cortisol secretion that is detected by pulses of circulating hormone levels which occur approximately every 60 min (Spiga, Walker et al. 2014). This basal pulsatile cortisol secretion depends primarily on the pituitary-adrenal closed loop with ACTH feedforward stimulation of glucocorticoid production, followed by subsequent feedback inhibition of corticotrope ACTH secretion. In humans, a prominent daily fluctuation of basal cortisol levels do occur, with maximal basal levels corresponding to the onset of the circadian active period (usually around awakening) (Spiga, Walker et al. 2014). This nychthemeral rhythm of cortisol excretion is supervised by neural signals from the supraoptic nucleus, which reach the PVN via local inputs, but also through splanchnic nerves directly acting on the adrenal gland (Charlton 1990). The PVN is a central area of the hypothalamus that integrates multiple inputs from several other brain regions, such as the brain stem, the midbrain, the limbic system and other hypothalamic nuclei (Gore 2008). Visceral information is received from catecholaminergic

pathways inputs coming from the brain stem, with important catecholaminergic arising from the nucleus of the solitary tract and the *locus coeruleus*. PVN inputs from the midbrain include somatic and special sensory information from the periaqueductal grey and pontine central grey regions. Another critical PVN connection is to the limbic system. Information on emotion and cognitive functioning is transmitted to the PVN hub by its extensive connections to the prefrontal cortex, septum, amygdala, hippocampus and bed nucleus of the *stria terminalis*. Therefore, in response to any form of perceived stress (internal or external) the PVN has a pivot role in initiating HPA axis activation (Sousa 2016). This arousal is a physiologic response to stress that overlaps the basal cortisol production and is intended to be of short duration, allowing the body to get the energy and drive needed to overcome the acute stressful stimuli in cause. If systematic repeated stress occurs, a chronic activation state of the HPA axis develops and can be deleterious to the immune, cardiovascular, neuroendocrine and central nervous systems (McEwen 1998).

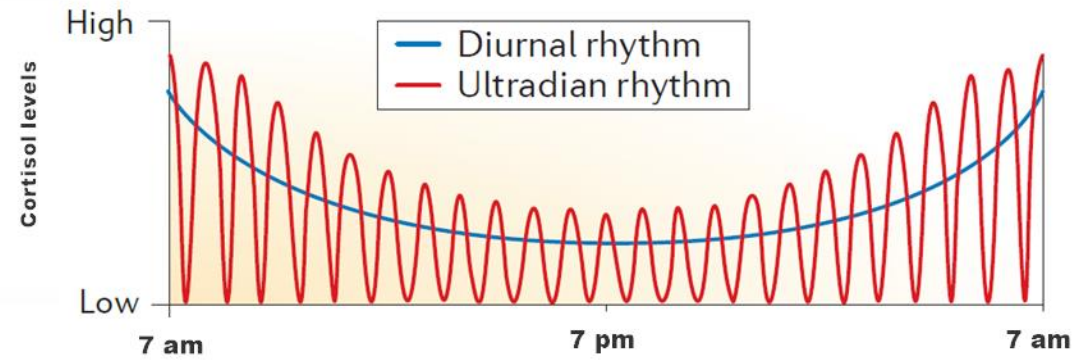

Figure 1.10. The diurnal human glucocorticoid rhythm (the circadian rhythm) varies greatly throughout the day (blue line), peaking at the beginning of the morning (awakening). The diurnal rhythm is an average of the ultradian rhythm (red line), which is created by cortisol pulses and has a much larger amplitude. Adapted from *Egeland, Zunszain et al. 2015* (Egeland, Zunszain et al. 2015).

Because of their lipophilic nature, most circulating cortisol molecules are bound to cortisol-binding globulin (transcortin), a specific protein carrier. About 20-50% of total plasma cortisol may be bound non-specifically to albumin and only 1-10% circulates unbound – free cortisol (Molina 2018). This free fraction is directly available for action and considered to be the biologically active portion of plasma cortisol. Adrenal steroid hormones trigger their physiologic action through genomic and

non-genomic pathways. Both gluco- and mineralocorticoid hormones bind to cytoplasmic receptors (belonging to the superfamily of ligand-activated transcription factors), form activated complexes that are then translocated to the cellular nucleus where the expression of genes containing hormone response elements is regulated. This genomic effect ultimately leads to altered levels of the corresponding proteins (Patel, Williams-Dautovich et al. 2014). Two nuclear receptors are involved: type I or mineralocorticoid receptor (MR) and type II or glucocorticoid receptor (GR)] (Molina 2018). MR have high affinity for both glucocorticoids and mineralocorticoids but is "protected" from cortisol by the enzyme 11β-hydroxysteroid dehydrogenase type 2, which rapidly inactivates nearby glucocorticoids (Gaillard and Bonenfant 2008). Therefore, cortisol effects target-tissues in a cell specific concentration and time-dependent mostly because of distinctive patterns of expression of these nuclear receptors and of the ability to metabolize glucocorticoids locally. Also of relevance, due to its much higher affinity for glucocorticoids, MR remains occupied even with very low concentrations of cortisol, while GR requires higher glucocorticoid levels to be activated which only happens during acute stress or during early morning cortisol peaks (Reul and de Kloet 1985). Adding to this complex mechanism, many distinct GR are formed by alternative splicing of the GR gene (NR3C1) (Barnes 2010). The two more relevant are: GRα - an "activating" nuclear receptor, and GRβ - the dominant negative one. Mutations, polymorphisms and alternative translation of this GR gene may help explain some of the perceived wide cellular glucocorticoid sensitivity (Quax, Manenschijn et al. 2013).

Non-genomic (rapid) actions have been also recognized probably linked to glucocorticoid membrane receptors. Although the nature and function of theses membrane receptors are largely unknown, they have been demonstrated to influence human immune cells through a specific protein product (annexin A1 – former called lipocortin) but also the hypothalamus, amygdala and prefrontal cortex *via* the endocannabinoid system (Hinz and Hirschelmann 2000, D'Acquisto, Perretti et al. 2008, Hill and McEwen 2010). Importantly, this non-genomic pathway seems to have an important role regulating the balance between pro-inflammatory and anti-inflammatory cytokines (Perretti, Ahluwalia et al. 1996). Many other physiologic systems are influenced by stress hormones, through both genomic and non-genomic actions. For example, throughout life, cortisol has an essential role on lung, liver and gut growth during foetal development and, after birth, it helps maintain the immune system response, the cardiovascular tone and relevant metabolic pathways (*e.g.* gluconeogenesis) (Molina 2018). It is estimated that, depending on the experimental

model, between 1 to 20% of all genes are regulated by glucocorticoids (Quax, Manenschijn et al. 2013).

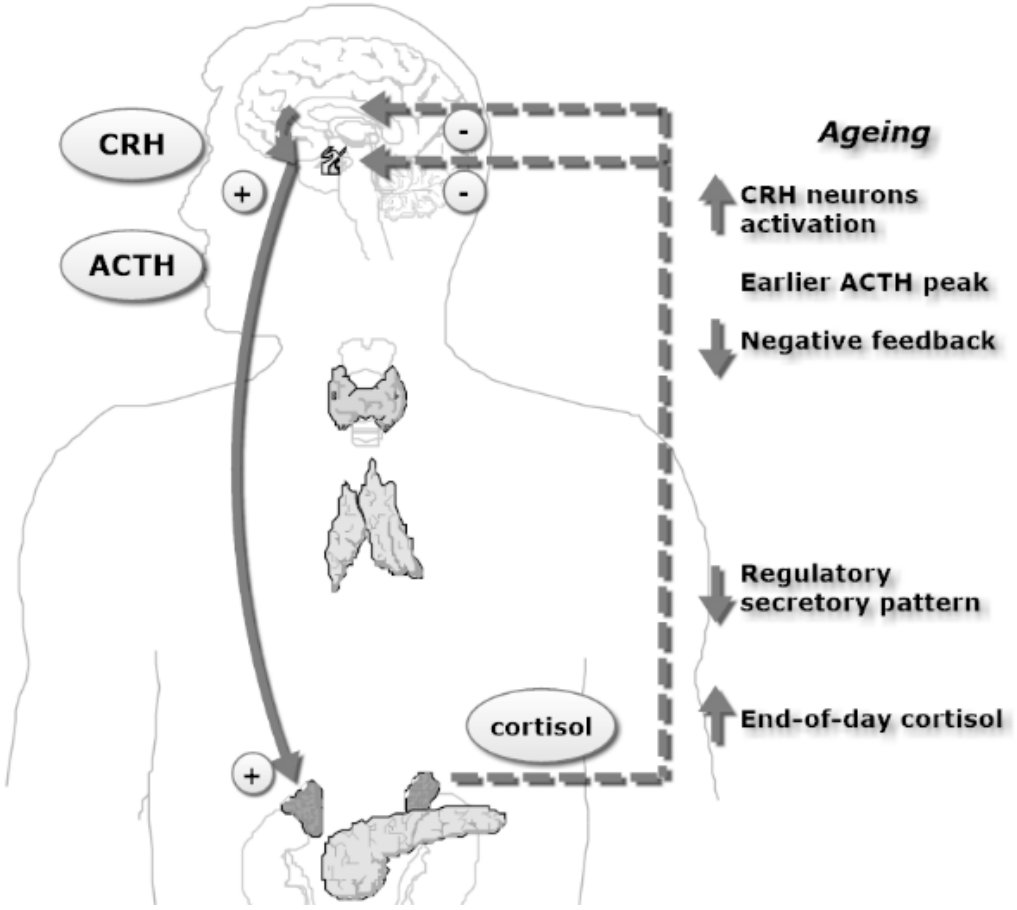

Figure 1.11. HPA axis and hormone secretion in ageing individuals. Adapted from *Veldhuis 2013* (Veldhuis 2013).

Defining HPA axis status

The most common measurements of HPA axis activity are the determinations of total cortisol and ACTH in plasma. As the main effector hormone of the HPA axis, cortisol levels seem to represent *the* key functional output of the axis. Yet, some important methodological limitations are to be considered: rapid HPA response to stress situations (*e.g.* venous puncture for blood collections), underlying presence of natural nychthemeral and ultradian rhythms, the very short blood half-life of HPA axis hormones besides cortisol (total cortisol: between 35-65 minutes; ACTH: below 15 minutes; CRH: less than 5 minutes) and the wide inter/intraindividual normal variance observed in plasma concentration of these hormones. These represent real challenges in HPA axis evaluation

(Schurmeyer, Avgerinos et al. 1984, Kraan, Dullaart et al. 1998, Spencer and Deak 2017). Therefore, the simultaneous morning plasma total cortisol and ACTH levels is considered a combined evaluation of the HPA axis status by determining pituitary and adrenal activation near its usual nychthemeral peak.

Determination of salivary free cortisol is a non-invasive way to determine individual cortisol tissue status almost instantly without the potential stress induced by the need for physical displacement and venous puncture (Turpeinen and Hamalainen 2013). It is an ultrafiltrate of plasma cortisol and reflects the biologically active levels, non-protein bound, of cortisol found in the plasma. It has been increasingly used to assess basal nychthemeral rhythm by obtaining evening (nadir) and early morning (peak) free cortisol levels (Harrison, Debono et al. 2019). The slope of plasma total or salivary free cortisol during awakening allows the measurement of normal cortisol awakening response (CAR), *i.e.* the rapid rise in cortisol that lasts 30–45 min immediately following morning awakening (Wilhelm, Born et al. 2007). The occurrence of this rising seems to reflect an adequate hippocampal function (Buchanan, Kern et al. 2004).

An easy and more prolonged analysis of the HPA activity involves urine sampling. In individuals without renal failure, concentration of urinary free cortisol reflects unbound and biologically active plasma cortisol (Spencer and Deak 2017). Twenty-four-hours urine samples are not affected by diurnal variation and indicate the full cumulative day cortisol production. A simple way of obtaining cortisol production during sleeping time is to collect first morning urine samples with determination of free cortisol-creatinine ratio (Burch 2011, Graham, Hunter et al. 2013). More recently, an extended and integrated measure of cortisol exposure (up to a period of several months) has been proposed by measuring hair cortisol concentration (Russell, Koren et al. 2012).

Another way of evaluating the HPA axis status is to perform suppressive/stimulating tests (*e.g.* insulin hypoglycaemia test, dexamethasone suppression test, etc.) (Tordjman, Jaffe et al. 2000, Nye, Grice et al. 2001). Due to their non-physiologic nature and to the diversity of available protocols, they are rarely used in population-based studies.

HPA axis and ageing

In general, ageing of the HPA axis produces important variations in its neural components. Aged adrenal glands may present a progressive androgen decline originating from the zona *reticularis* (*e.g.* dehydroepiandrosterone – DHEA). All other hormonal changes are almost exclusively the

result of altered hypothalamic and pituitary secretion. DHEA reduction (above 20% of peak values in men and 30% of peak values in women around age of 70 to 80 years) is, by some, assumed to define *adrenopause* even when not accompanied by a decrease in the concentration of other adrenal cortex hormones (Hornsby 1995, Ravaglia, Forti et al. 1996).

Extensive work has been done exploring the effects of age in the HPA axis. In animal models, senescence was associated to a loss of cortisol circadian rhythmicity, predominantly due to a reduction in pulse amplitude during the peak phase, but also linked to an overall decline of ultradian pulsatility throughout the nychthemeral cycle (Spiga, Walker et al. 2014). A functional decrement of glucocorticoid negative feedback loop with an increased activation of CRH and AVP neurons, and higher hypothalamic-pituitary CRH portal levels (Hauger, Thrivikraman et al. 1994, Swaab and Bao 2011) were also associated with aging, although not consistent among various species . The precise location within the HPA axis in which aging begins is not yet known. Some authors propose hippocampal oxidative damage and a reduction of neurons expressing GRs and MRs in the CA1 hippocampus area as the primordial event (Morano, Vazquez et al. 1994, Sato, Takahashi et al. 2010). This incident will begin the adverse "glucocorticoid cascade" process (see below), where long-term cortisol hypersecretion will in turn increase susceptibility of the hippocampus and PFC to damage, further enhancing activation of the HPA axis and prolonging the hippocampus increased-susceptibility status (Sapolsky, Krey et al. 1986, Cerqueira, Mailliet et al. 2007).

In humans, the natural response to adverse or stressful stimulus by the HPA seems to be influenced by age, but the direction of the change is not consistent (Van Cauter, Leproult et al. 1996, Purnell, Brandon et al. 2004, Roelfsema, van Heemst et al. 2017). A tendency towards an overall increase of 24-hour cortisol production and an earlier morning cortisol peak (phase advance) is observed in older individuals (Van Cauter, Leproult et al. 1996, Heaney, Phillips et al. 2012). However, whether this advance phase reflects a true circadian shift or just behavioural differences between younger and older adults (*e.g.* earlier bedtime and sooner waking hour) is still to be clarified (Liyanarachchi, Ross et al. 2017). Despite relative preservation of diurnal rhythmicity on cortisol secretion during ageing, nychthemeral rhythm of ACTH and cortisol concentrations seem to be blunted in absolute amplitude due to an increased in *nadir* levels in older individuals (Sherman, Wysham et al. 1985, Van Cauter, Leproult et al. 1996). It is not clear if this late-day increase in cortisol levels simply reflect the circadian phase advance observed in many ageing individuals (Liyanarachchi, Ross et al. 2017). Interestingly, studies carried out on centenarians, and near as old individuals, have shown that this age group displays a more dynamic activity of

the HPA axis (*i.e.* greater cortisol fluctuations and diurnal decline). Interestingly, a lower 24-hour cortisol secretion was associated with better physical performance (Ferrari, Cravello et al. 2008, Gardner, Lightman et al. 2013). An age-related HPA state of hyper-responsiveness has also been described. CRH levels and certain amplifiers of ACTH and/or cortisol response seem to be more prominent in older age groups (Born, Ditschuneit et al. 1995, Veldhuis, Sharma et al. 2013). Exaggerated acute pituitary responses to stress in these ageing individuals may point to a reduced negative HPA axis feedback (Seeman, Singer et al. 1995). Any relative decrease in density of MR and GR receptors in the hippocampus, which has already been described in older adults, could explain this impaired negative feedback, overall elevated cortisol secretion and weakened cortisol suppression by dexamethasone (O'Brien, Schweitzer et al. 1994, Wilkinson, Peskind et al. 1997). However, the underlying basis for any age-dependent dysfunction in the glucocorticoid allostatic performance is still not fully understood (Vitale, Salvioli et al. 2013). Care should be taken when interpreting these experimental observation in the HPA axis performance during ageing since most studies do not control for common confounders such as sex, body weight, sleep pattern or chronic diseases (Andrew, Phillips et al. 1998, Roelfsema, Pijl et al. 2012, Veldhuis 2013).

HPA axis and cognition

As one of the main survival stress effectors, adrenal steroids are of primordial importance for brain function and development [reviewed by (Sousa and Almeida 2002, McEwen, Bowles et al. 2015)]. Since the first recognition of corticosteroid receptors in the rat brain in the 1960's, considerable research has been published on the topic (McEwen, Weiss et al. 1968, Koning, Buurstede et al. 2019). Today, it is recognized that these receptors are expressed globally over human brain with a distribution that varies according to its sub-type (de Kloet, Joels et al. 2005). MRs are highly expressed in hippocampal–limbic area and, by contrast, GRs have a more extensive distribution englobing the pituitary, various areas of the hypothalamus, thalamus, raphe area, prefrontal and other cortex regions (Reul and de Kloet 1985, Sanchez, Young et al. 2000). Due to the lack of 11β-hydroxysteroid dehydrogenase type 2 expression in the hippocampus and other brain areas, except for the nucleus of the solitary tract and circumventricular neurons, MR could be considered almost as a purely glucocorticoid receptor within the central nervous system (de Kloet, Meijer et al. 2018). The selective action of MR or GR by adrenal steroids have shown to provoke a neuron biphasic activation (Diamond, Bennett et al. 1992, Pavlides, Watanabe et al. 1995). As stated before, MRs have a high glucocorticoid affinity while GRs have a low glucocorticoid affinity, meaning that the

later are only activated in the presence of high cortisol levels (like during the peak of normal circadian rhythm or after stress) (Joels 2006). Therefore, oscillations of glucocorticoids may determine GR activation and deactivation, while constant MR occupancy provides a more stable neuron excitability (Stavreva, Wiench et al. 2009). Persisting elevated levels of glucocorticoids during early, but also late, development stages have been linked to structural brain abnormalities with potentially negative effects on overall cognition capacities (Sousa and Almeida 2002, Mesquita, Wegerich et al. 2009, Li, Wu et al. 2014).

Animal studies

More than thirty years ago *Sapolsky et al.* proposed that persistent elevations of glucocorticoids (even within physiological levels) would impair hippocampal neurons capacity to survive extrinsic challenges through abrogation of hippocampal-HPA axis feedback inhibition (Sapolsky, Krey et al. 1986). This occurrence would precipitate further damage to the feedback loop and intensify hippocampal neurons loss – the "glucocorticoid cascade" hypothesis (Sapolsky, Krey et al. 1986). This theory was originally confirmed by the observation that, in animal models, chronic stress or excess of exogenous glucocorticoids, especially during key moments of brain development, could cause permanent loss of hippocampal neurons, as a result of increased neuronal death and/or decreased neurogenesis (Mizoguchi, Kunishita et al. 1992, Cameron and Gould 1994, Gould, Tanapat et al. 1998). Accordingly, age-associated hippocampal atrophy was also presented as an hypothesis for the decline on the ability to control cortisol secretion (*via* hippocampus) observed in ageing (Sapolsky 2000). Additionally, excess of glucocorticoids was associated to reversible neural remodelling with retraction of neuron apical dendritic branches in the hippocampus (CA3 and DG regions) and in the medial prefrontal and anterior cingulate cortex, among others (Magarinos and McEwen 1995, Cerqueira, Catania et al. 2005, Cerqueira, Pego et al. 2005, McEwen, Nasca et al. 2016). Interestingly, in rats, aging was associated with a loss in the ability to re-extend dendrites after cessation of the stress stimulus in the medial PFC (Bloss, Janssen et al. 2010). More recent studies in non-human primates using different psychosocial stressors, as well as others in rodents using distinctive steroid receptor stimulation and more "physiologic" protocols, have failed to confirm these negative findings linked to glucocorticoid excess (especially the neuronal loss features) and their relevance in the HPA axis functioning (Vollmann-Honsdorf, Flugge et al. 1997, Sousa, Paula-Barbosa et al. 1999). Currently, it is recognized that the hippocampal "glucocorticoid

cascade" hypothesis should be rather integrated with other neuro-anatomical players for the control of the HPA axis (*e.g.* bed nucleus of the stria terminalis, amygdala and endocannabinoid system), as well as with many other influences such as genetic risk, early life programming and distinctive individual structural brain plasticity response to GR and MR ligands (Tasker and Herman 2011, Lucassen, Pruessner et al. 2014). These potentially reversible and non-reversible brain microstructural fluctuations induced by glucocorticoids can be translated into some brain macrostructural changes. For example, consistent reductions on rat brain volume were observed by imaging techniques in areas described above as "sensitive" to endogenous and exogeneous glucocorticoids (*i.e.* hippocampus and PFC) (Cerqueira, Catania et al. 2005, Schubert, Kalisch et al. 2008).

Human studies – brain morphology and function

There is some controversy about the true effects of corticoids in the human brain, probably due to the limited number of studies available and to the difficulty in performing such studies. Several authors evaluating the consequence of acute corticosteroid exposure on human brain architecture did not observed hippocampal volume change in healthy adults (from single administration up to 4-days use) (Tessner, Walker et al. 2007, Scheel, Strohle et al. 2010). On the contrary, authors like *Brown et al.* observed an overall 1.7% hippocampal volume reduction, more pronounced in women, after only 3 days of high dose hydrocortisone (160 mg per day) (Brown, Jeon-Slaughter et al. 2015). As for chronically elevated levels of corticosteroids, data is available from many human observational studies. Chronically depressed patients (frequently used as a model of HPA axis "hyperfunction") have shown signs of hippocampal dysfunction, including 12-15% decrease in hippocampal volume (Sheline, Wang et al. 1996). Similar imaging results were obtained from patients with Cushing syndrome, chronically stressed or on chronic oral glucocorticoids, all of them reporting 8-9% hippocampal volume reductions (Starkman, Gebarski et al. 1992, Brown, D et al. 2004, Gianaros, Jennings et al. 2007). In these studies, the extent of hypercortisolism seems to correlate with the degree of hippocampal volume loss. Some reversibility has also been shown to occur after reverting hypercortisolism, as seen in a cohort of patients with Cushing's disease where a mean 10% volume recovery was found after surgical treatment (Starkman, Giordani et al. 1999). The explanation for these hippocampal volume fluctuations is not straightforward and its reversibility has been recently questioned (Frimodt-Moller, Mollegaard Jepsen et al. 2019). These

rapid volume regain cannot be fully explained by any sort of modulation in neuronal death or neurogenesis, as proposed and observed in rodent's hippocampus (Reagan and McEwen 1997, Saaltink and Vreugdenhil 2014). Some other "fast-responder" mechanisms must be present. There is the possibility of a decrease in cellular water content, as seen after systemic exposure to glucocorticoids in the treatment of brain oedema, but the specificity of volume reduction seen in hippocampus (*versus* no change in other brain areas) suggests a distinct process, possibly mediated by GR receptors (Chumas, Condon et al. 1997). As discussed above, within the rodent's hippocampus, chronically high levels of glucocorticoids were found to induce regression of neuron apical dendritic branches, which could help explain some of the overall brain volume reductions (Sapolsky, Uno et al. 1990, McEwen, Nasca et al. 2016). Nevertheless, no quick effect on global dendrite arbor and volume was detected with acute administration of glucocorticoids in rats, despite of an increased in spinogenesis observed in the hippocampus (Liston and Gan 2011, Yoshiya, Komatsuzaki et al. 2013).

In elderly humans, higher levels of salivary cortisol in the evening were associated to smaller total brain volumes, especially due to grey matter loss (Geerlings, Sigurdsson et al. 2015). Similar morphologic changes were identified in clinical models of chronic hypercortisolemia such as in Cushing's disease patients. In some studies, progressive reductions of hippocampus volume were related to illness duration rather than age; these were sometimes reverted after surgical treatment and "normalization" of the HPA status (Starkman, Gebarski et al. 1992, Sheline, Sanghavi et al. 1999, Starkman, Giordani et al. 1999). On the contrary, other studies in the elder associated higher morning cortisol to slightly greater white matter volumes (Geerlings, Sigurdsson et al. 2015). In human stress-HPA activation models, such as post-traumatic stress disorder patients, evening salivary cortisol was also correlated to overall reduction in brain cortical volume but not to hippocampal or amygdala changes in size (Babson, Woodward et al. 2017). These potential distinctive effects of stress or high cortisol levels over distinct regional brain areas known to be enriched on GRs expression, may be explained by some important physiological distinctions between a simple HPA activation *versus* a full stress-response.

Table 1.7. Key points about HPA, cortisol and brain function.

- GR has an extensive presence over the brain while MR are almost restricted to hippocampal-limbic areas
- High levels of cortisol are associated to reduced adult total brain volume (hippocampus more vulnerable?)
- The most consistent human neuro-cognitive domains influenced by serum cortisol levels is Attention and Memory
- Contrasting cognitive outcomes are present in response to acute or chronic exposure to high levels of cortisol

Human studies – neurocognitive performance

Several studies have searched for a link between structural hypercortisolism and abnormalities on functional brain and neurocognitive measures, especially memory. Many authors have evaluated the interaction of the HPA axis and cognition during healthy ageing. Early works described a negative impact of high plasma (and urinary) cortisol levels on memory performance (Seeman, McEwen et al. 1997, Lupien, de Leon et al. 1998). However, these studies were mostly done with small samples, some of them including participants with preclinical dementia and therefore potentially biased by HPA axis hyperactivation already present before progression to fully blown clinical dementia. Larger and better-designed studies were since then published exploring several aspects of cognition. It is now clearer that glucocorticoids can both facilitate and suppress memory processes depending on circulating levels, acute or chronic exposure and stimulus-response temporal connexion. More recently, an U-shape curve was proposed to explain a link between various cognition measures and glucocorticoid levels, where too much or too long exposure appears to be detrimental to brain-sensitive areas, like the hippocampus, PFC and amygdala (Joels 2006). This hypothesis would help clarify the reduction of some related cognitive processes including memory, executive function and mood, observed in the extremes of HPA axis activation. An important issue not fully elucidated relates with the possibility that ageing, *per se*, could turn individual neurocognitive features more susceptible to HPA axis dysfunction (in either direction) (Bodnoff, Humphreys et al. 1995). It is also not clear whether this vulnerability is associated to the HPA axis blunted response observed in some older subjects (see above).

In young subjects, glucocorticoids seem to enhance the consolidation of new memories while reducing the process of retrieval of previously stored information (Buchanan and Lovallo 2001, Wolf, Atsak et al. 2016). In this age-group, acute stress glucocorticoid response (moderate intensity

and within the context of learning) has also produced a positive cognitive effect on focused attention and memory (Yuen, Liu et al. 2009). In older individuals, most observational studies have linked nocturnal HPA axis hyperactivation and increased basal cortisol levels to poorer cognitive performance. Again, some of the studies included individuals with preclinical dementia (Seeman, McEwen et al. 1997, Lupien, de Leon et al. 1998) and, as such, potentially overestimate this negative association. Larger and better designed studies were published since then, exploring several aspects of cognition. In a 4,244 Icelandic cohort (mean age 76-yr) without dementia, a poorer cognitive functioning across several domains (memory, speed processing and executive functions) was associated to higher evening salivary cortisol levels (Geerlings, Sigurdsson et al. 2015). In the same report, higher levels of morning cortisol were only associated with slightly better processing speed and executive functioning, but not with memory performance. On the contrary, in a prospective study of 97 non-depressed elderly men (age 65-70 years), elevated plasma morning cortisol was related with poorer general cognitive performance status and working memory and episodic (verbal) memory tasks (MacLullich, Deary et al. 2005). In the Baltimore Memory Study (1,140 participants aged 50-70 years) a higher mean diurnal salivary cortisol was related to worse performance in 6 different neurocognitive domains (language, processing speed, eye-hand coordination, executive functioning, verbal memory and learning, and visual memory) independently of age, sex, education, ethnicity, time of the day and other covariates (Lee, Glass et al. 2007). Consistent with the later study, in the Longitudinal Aging Study Amsterdam (with 1,154 participants aged 65-yr or more), higher diurnal cortisol levels at baseline were also associated to poorer memory function and speed of information processing (Comijs, Gerritsen et al. 2010). In the MacArthur Field Study, increased overnight cortisol production was only found to be independently related to poorer baseline memory performance in women (total of 1,313 subjects aged 70-79 years) (Seeman, McEwen et al. 1997). This gender effect was assumed to be linked to a somewhat smaller neurocognitive changes observed in men and was not reported in follow-up studies (Karlamangla, Singer et al. 2005). Yet, more recently, in a community-dwelling young and middle-aged adults without dementia cohort of more than 2,000 individuals, *Echouffo-Tcheugui et al.* found that higher morning cortisol was associated with impaired memory in healthy younger to middle-aged adults, with a more evident association in women (Echouffo-Tcheugui, Conner et al. 2018). Evaluation of the cortisol awakening response slope also provided mixed results. Elevated cortisol awakening responses were associated to better working memory and executive functioning by some authors (Almela, van der Meij et al. 2012, Evans, Hucklebridge et al. 2012). Others did

not describe such findings and reported only a positive effect on episodic memory (Franz, O'Brien et al. 2011, Ennis, Moffat et al. 2016). All these data seem to indicate an important connection between recent cortisol exposure and memory domain tasks, particularly in women.

A more prolonged analysis of the individual HPA status has been of interest to many authors. In The Vietnam Era Twin Study of Aging, 15 salivary cortisol samples, paralleling individual diurnal rhythms across 3 non-consecutive days, were obtained in a subgroup of 778 twin men aged 51-60 years with several cognitive domains being assessed through 24 standard measures that included general cognitive ability, verbal and visual-spatial memory, short-term/immediate memory, working memory, executive function, verbal fluency, abstract reasoning and psychomotor processing speed (Franz, O'Brien et al. 2011). Higher levels of average area-under-the-curve cortisol output across the 3 days related with poorer performance in executive (primarily set-shifting) measures, processing speed and visual-spatial memory. In accordance with this observation, higher mean and diurnal area-under-the-curve salivary cortisol levels were also associated to worse attention and short-term verbal memory in a 57 healthy older Spanish population (mean age 65-yr) (Pulopulos, Hidalgo et al. 2014). In contrast, these same authors reported that lower hair cortisol concentrations (used as a long-time cortisol secretion measure) were constantly associated to worse working memory and both short or long-term verbal memory, suggesting that individuals with less chronic cortisol exposure may be more cognitively vulnerable to any more "acute" HPA axis negative dysregulation. The relationship between cognitive decline and the HPA axis status during ageing was approached by few longitudinal studies, with some inconsistent data reported. Baseline morning plasma cortisol or any other diurnal salivary cortisol secretion pattern were not associated with the cognitive performance or decline in a Dutch and British 5-6 years follow-up cohorts (Comijs, Gerritsen et al. 2010, Singh-Manoux, Dugravot et al. 2014). In a lifelong longitudinal study started in Scotland in late 1940's the authors also found little evidence for a significant role of cortisol secretion on the cognitive performance over a period of 60-year follow-up (370 participants with a mean 78-yr of age at cortisol sampling) (Harris, Cox et al. 2017). Interestingly, they reported that a flatter diurnal slope was linked with a somewhat greater lifelong relative cognitive decline, a feature that was already stated by some other shorter studies addressing longitudinal memory performance (Beluche, Carriere et al. 2010). Some authors studying patients with Alzheimer disease did not find differences inn HPA axis activation between more cognitive impaired and the control age-matched healthy population (Swaab, Raadsheer et al. 1994). Longitudinal studies also failed to show a coherent link between overall

higher evening cortisol levels and cognition (see above). These results and the ones coming from observational studies with Cushing's disease patients appear to contradict the proposed thesis of the "glucocorticoid cascade" hypothesis and the associated cognitive decline (Muller, Lucassen et al. 2001).

Intervention trials

To explore further the role of corticoids on cognition, some randomized *intervention trials* have been published exploring acute effects of gluco- and mineralocorticoid stimulation/suppression on neurocognitive abilities in healthy and in depressed individuals. Acute hydrocortisone administration in healthy young subjects was found to alter attention to emotional stimuli and to enhance consolidation of new memories, but not to influence significantly cognitive flexibility (Buchanan and Lovallo 2001, Vaz, Pradella-Hallinan et al. 2011, Wingenfeld, Wolf et al. 2011, Breitberg, Drevets et al. 2013). Adding some more complexity, recent reports showed that some of the positive fast (non-genomic) effects over cognitive flexibility observed just minutes after hydrocortisone administration are largely dependent on the basal cortisol levels (Dierolf, Arlt et al. 2016). This result highlights the difficulty on interpreting the various studies published on this subject. Mineralocorticoid receptor blockage with spironolactone (with the simultaneous glucocorticoid receptor activation by its enhancing cortisol secretion counter-effect) was shown to impair attention in non-stressed subjects and to enhance long-term memory (while reducing working memory performance) in stressed individuals (Cornelisse, Joels et al. 2011). Glucocorticoid receptor blockade with mifepristone was found to improve cognitive function in bipolar depressed patients, potentially due to an increased mineralocorticoid receptor effect (Watson, Gallagher et al. 2012). In healthy young individuals, blockage of the MR impaired memory retrieval while GR blockage enhanced it (Rimmele, Besedovsky et al. 2013). Mineralocorticoid receptor stimulation with fludrocortisone was shown to have positive effects on verbal memory and executive function in depression and in elderly healthy subjects (Hinkelmann, Wingenfeld et al. 2015, Otte, Wingenfeld et al. 2015). These findings suggest a somewhat opposing role of MR and GR on cognitive tasks (especially in memory retrieval). Cortisol levels appear to be related to optimal cognitive performance in an inverted U-shape, indicating that intermediate cortisol levels, by activating MR in prefrontal and hippocampal neurons and at the same time leaving GR mostly unoccupied, are indeed beneficial to brain functioning. Just recently, another relevant finding was

published after testing three different methods of hydrocortisone replacement in healthy non-stressed male volunteers when the authors observed that distinct patterns, or rhythms, of hydrocortisone administration had the possibility of producing diverse impacts over human cognition (Kalafatakis, Russell et al. 2018). The main finding being that the group treated with a less "physiological" ultradian time-set cortisol rhythm presented poorer working memory performance, particularly when exposed to increased cognitive challenges.

Some reviews and *meta-analyses* addressed the relation between performance in neurocognitive tasks and acute cortisol administration. In 2005, *Het et al.* analysed 16 studies on the cortisol effect over memory (Het, Ramlow et al. 2005). They have found a significant decrease in declarative memory performance when cortisol was administered just before memory retrieval. No effect was consistently found when cortisol was administered before the learning task. Interestingly, the included experiments have shown that cortisol administration had different effects depending on the time of the day chosen for administration. Morning administration induced memory impairment whereas studies conducted during the afternoon showed a small, but significant, memory performance improvement. The possibility that this finding translates differential activation of MR and GR during the circadian cycle could help corroborate the diurnal variances also found in some neurocognitive performances (and their susceptibility to cortisol action) reported by several observational studies (see above) (Hidalgo, Almela et al. 2016).

Another recent *meta-analysis* was published addressing cortisol effect over core executive functions. This report included 18 studies evaluating working memory, 23 assessing inhibition and 6 analysing set-shifting abilities in healthy participants (Shields, Bonner et al. 2015). The overall result was non-significant for all these three executive function components, with no age or gender modulation effect observed. Substantial heterogeneity between existed in determining working memory, but when separating rapid from delay effects of cortisol administration, cortisol impaired working memory only if participants were tested between 15 to 73 min after cortisol administration. After 74 min it actually led to improvement with no dose effect detected. Reports evaluating inhibition were more homogeneous but a significant positive effect with improved tasks was only detected between 15 to 135 minutes after cortisol supply. For set-shifting after acute cortisol administration, no differential effect over time was found.

Although all included studies used fast "acute cortisol increase" protocols they validate data coming from many other studies addressing stress, where acute response was associated to enhancement in inhibition and to impairment of working memory (Schoofs, Preuss et al. 2008, Schwabe, Hoffken et al. 2013, Shields, Sazma et al. 2016). Chronic exposure to high levels of cortisol is associated to worse executive functions in animal models and in patients suffering from hypercortisolism conditions, but only a few studies have assessed experimental chronic cortisol administration in humans with mixed results regarding working memory (Newcomer, Selke et al. 1999, Young, Sahakian et al. 1999, Cerqueira, Pego et al. 2005, Forget, Lacroix et al. 2016, Ragnarsson, Stomby et al. 2017).

To summarize, despite some evidence of a beneficial effect of intermediate levels HPA activation on memory domains, available data in older cohorts is not so clear into what extent memory, executive functions and/or overall cognitive decline seen during senescence, is actually modulated by the ageing HPA axis and particularly if it responds to chronic or *de novo* excessive cortisol exposure.

The following table attempts to summarize studies addressing the association of HPA activity and neurocognitive performance.

Table 1.8. Relation between Cortisol secretion and distinctive neurocognitive features.

Neurocognitive feature		Cortisol relationship	Reference examples
Global cognition			
		NEUTRAL (morning)	Comijs, Gerritsen et al. 2010;
		NEGATIVE (evening)	Lupien, de Leon et al. 1998;
Cognition decline during ageing		NEUTRAL	Singh-Manoux, Dugravot et al. 2014
Executive function			
	Working memory	INVERSE (evening)	Stawski, Almeida et al. 2011;
		INVERSE (morning)	MacLullich, Deary et al. 2005;
		POSITIVE (morning)	Almela, van der Mei jet al. 2012;
	Set shifting	INVERSE (evening)	Geerlings, Sigurdsson et al. 2015;
Memory			
	Semantic	INVERSE (evening)	Seeman, McEwen et al. 1997
	Verbal	INVERSE (morning)	Comijs, Gerritsen et al. 2010;
		POSITIVE (morning)	Ennis, Moffat et al. 2016;
	Visual	INVERSE (morning)	Franz, O'Brien et al. 2011;
Attention		POSITIVE (acute) NEGATIVE (chronic)	Yuen, Liu et al. 2009;

1.7 Hypothalamus-pituitary-thyroid axis

The hypothalamus-pituitary-thyroid (HPT) axis is comprised by thyrotropin-releasing-hormone (TRH) neurons from the paraventricular nucleus (PVN), thyrotrope cells from the pituitary and follicular cells from the thyroid gland, where thyroid hormones (TH) are finally produced and secreted. Phylogenetically, it is one of oldest vertebrate endocrine systems with a primitive thyroid gland structure found in the pharyngeal area of adult cyclostomes, although not under complete pituitary regulation (David Norris and Carr 2013).

In humans, thyroid hormone synthesis and release is primarily under negative feedback control by the HPT axis. Parvicellular neurons from PVN secrete TRH within the median eminence and into the primary plexus of the pituitary portal circulation. This secretion is modulated through retroactive negative feedback from thyroid hormones, several input neuronal circuits (essentially *via* arcuate nucleus) and other neuroendocrine effectors (*e.g.* glucocorticoids, dopamine, somatostatin, norepinephrine) (Molina 2018). At the anterior pituitary lobe, thyrotropes respond to this TRH by increasing thyrotropin (thyroid-stimulating-hormone, TSH) synthesis and release into the systemic circulation. At the thyroid gland, TSH will activate all the steps involved in TH production, including iodine uptake and organification, thyroid growth and TH synthesis, storage and release. There are primarily two forms of TH: thyroxine (T_4) and triiodothyronine (T_3). T_4 is exclusively produced by follicular cells of the thyroid gland, whereas most T_3 is produced peripherally by deiodination of thyroxine. T_3 is the main active form of TH and its plasma concentration is largely dependent on the presence of the "prohormone" T_4 and its deiodination in liver and muscle tissue. The thyroid gland also works as a warehouse and holds large quantities of T_4 and T_3 incorporated into thyroglobulin – the main protein found in follicular colloid that acts as a scaffold for TH synthesis. Being stowed in this way, the thyroid gland is able to store more than 2 month's supply of T_4/T_3 (as well as of iodine) and to respond quickly to any TSH surge (Molina 2018). Adding some complexity to this "classical" feedback construction, other physiological HPT features have been recently uncovered, such as the "ultrashort" *autocrine loop* (where TSH inhibits its own secretion at the pituitary level), and the *TSH-T_3 shunt* (representing intrathyroidal T_3 production through a direct response to TSH) (Dietrich, Midgley et al. 2018).

In subjects with normal night sleep cycles, TSH secretion follows a basal nycthemeral pattern with an acrophase between midnight and 05h a.m. and nadir levels around noon (Roelfsema and

Veldhuis 2013). In contrast to some other pituitary hormones with circadian secretion patterns, the nocturnal increase of TSH precedes the onset of sleep and is insensitive to physiological suppression (Roelfsema, Pijl et al. 2014). Superimposed over this basal secretion, euthyroid individuals also present TSH pulses, approximately every 2-3 hours, which will account for around 60-70% of the total daily secretion (Samuels, Veldhuis et al. 1990). Despite the influence of this circadian rhythm and TSH bursts on TH secretion, its effect on T_3 and T_4 blood concentration is somewhat negligible (resulting in less than 12% variation from nadir to peak), perhaps due to the TH long plasma half-life (Russell, Harrison et al. 2008).

T_4 and T_3 are lipophilic molecules so, once released into the circulation, most will bind reversely to proteins (around 70% to thyroid-binding-globulin, 10% to transthyretin and 15-25% to albumin). The small fraction (<0.1%) of both hormones that circulates free (FT_3 and FT_4) is considered the biological "active" fraction that reaches cells (Molina 2018). These binding proteins have a buffer and storage function that helps maintain serum FT_4 and FT_3 concentrations within narrow limits and ensures its immediate and continuous supply to tissues. Despite their lipophilic nature, several transporters have been identified in the plasma membrane and are critical for the transfer of thyroid hormones into target cells (Heuer and Visser 2009). Disparity on tissue expression has been documented among the three main families of transmembrane thyroid hormone transporters – monocarboxylate transporter (MCT), organic anion transporting polypeptide (OATP) and L/type amino acid transporter (LAT) (Bianco, Dumitrescu et al. 2019). It is now recognized that some of these transmembrane proteins, like the monocarboxylate transporter 8 (MCT8) and the organic anion transporting polypeptide 1C1 (OATP1C1), are especially critical for brain transport of T_3 and T_4, respectively (Brockmann, Dumitrescu et al. 2005, Mayerl, Muller et al. 2014, Groeneweg, Visser et al. 2017).

Almost the entire actions of TH are initiated by binding and activating specific nuclear receptors (Mendoza and Hollenberg 2017). These thyroid hormone receptors (TR) are transcriptionally active proteins present in virtually all tissues that dynamically control the expression of many thyroid hormone–responsive genes (Cheng, Leonard et al. 2010). Thyroid hormone receptors (as other class II nuclear receptors) have the capacity of binding to specific areas of DNA both in the presence or absence of TH (Tsai and O'Malley 1994). Many of these sequences, known as thyroid hormone-response elements, consist of two tandemly arranged hexamer motifs with a consensus sequence to which the high T_3 affinity (but low capacity) TR/retinoid X receptor (RXR) heterodimers binds (Oetting and Yen 2007). The DNA-bound ligand-free TR interacts with corepressors which will result

in stopping target gene transcription. The genomic action of T_3 starts by inducing a conformational change of TR, therefore facilitating corepressor dissociation and promoting transcription of thyroid hormone–responsive genes (Oetting and Yen 2007). There are 2 major subtypes of TR (TRα and TRβ) and several isoforms obtained by alternative splicing (TRα1, Trα2, Trα3, TRβ1, TRβ2, TRβ3 and TRβ4) (Brent 2012, Anyetei-Anum, Roggero et al. 2018). Their subtype and isoform expression is tissue-dependent and regulated differently throughout development and ageing (Cheng 2000). For example, cardiovascular effects mediated by T_3 are mainly dependent on the TRα1, whereas those linked to pituitary function and differential retinal cone photoreceptors development are mostly reliant on TRβ2 isoforms (Brent 2000, Anyetei-Anum, Roggero et al. 2018, Eldred, Hadyniak et al. 2018).

Non-genomic actions of TH were first recognized more than 30 years ago when activation of Ca^{2+}-ATPase by $T_4$and T_3 was demonstrated in enucleated cells (Davis, Cody et al. 1983). It is currently recognized that some of these fast-action responses begin with some G-protein-coupled receptors found in cell membrane and cytoplasm that share structural homologies with nuclear thyroid hormone receptors (like truncated TRα isoforms) and mediate T_3 actions *via* downstream targets such as mTOR or Rac GTPase (Cao, Kambe et al. 2005, Storey, Gentile et al. 2006). Some other fast TH responses appear to rely on unrelated TR targets, such as integrin αvβ3, which mediates T_4 action by activation of phospholipase C (PLC), protein kinase C (PKC) and mitogen activated protein kinase (MAPK)1 and MAPK2 cascades (Davis, Goglia et al. 2016). More notably, TH non-genomic regulation of cellular respiration in mitochondria is vastly dependent on the commonly expressed mitochondrial receptor p43, that in response to T_3 increases both mitochondrial transcription and protein synthesis (Wrutniak-Cabello, Casas et al. 2001). Presently, all these signalling mechanisms and non-genomic control of gene expression have been only partially elucidated and often confused with some "direct" genomic TH actions (Hammes and Davis 2015).

Three deiodinase enzymes are responsible for TH metabolism. They present distinct tissue distribution, catalytic profile, physiologic function and regulation (Marsili, Zavacki et al. 2011). In rodents, type 1 iodothyronine deiodinase is the main enzyme responsible for T_4 conversion to T_3, but it is also able to perform its inactivation by converting T_4 to rT_3 and T_3 to T_2. In humans, this somewhat kinetically ineffective enzyme (which can catalyse both activation of T_4 by outer-ring deiodination and inactivation by inner-ring deiodination), is mainly expressed in the liver, kidney and thyroid, with an unclear overall physiological role (Gereben, Zavacki et al. 2008). Type 2

iodothyronine deiodinase is highly expressed in the brain (mainly in glial cells), pituitary, brown adipose tissue, heart and skeletal muscle. Its key role is to activate locally T_4 into T_3 and to provide intracellular T_3 "on demand" supply (Bianco and Kim 2006, Gereben, Zeold et al. 2008). Type 3 iodothyronine deiodinase is the main TH inactivating enzyme, converting T_4 to reverse-T_3 and T_3 to T_2. It is present during early foetal life in the placenta but also highly expressed in adult skin and brain tissue (mostly in neurons), where it is thought to protect the brain from potentially excessive TH plasma concentrations (Ng, Hernandez et al. 2009, Marsili, Zavacki et al. 2011).

Due to its ubiquity and intricate mechanism, it is easy to recognize that TH are essential for the development and maintenance of many of the human biological systems. They have a critical role in various cellular mechanisms such as transcription of Na/K-ATPase and oxygen consumption; protein synthesis and degradation, essential to growth and development; heat generation; gluconeogenesis; cholesterol synthesis; and other metabolic pathways. Some important TH processes also depend on non-TH signal transduction pathways, such as adrenergic signalling and other metabolism-linked hormone systems (Brent 2012).

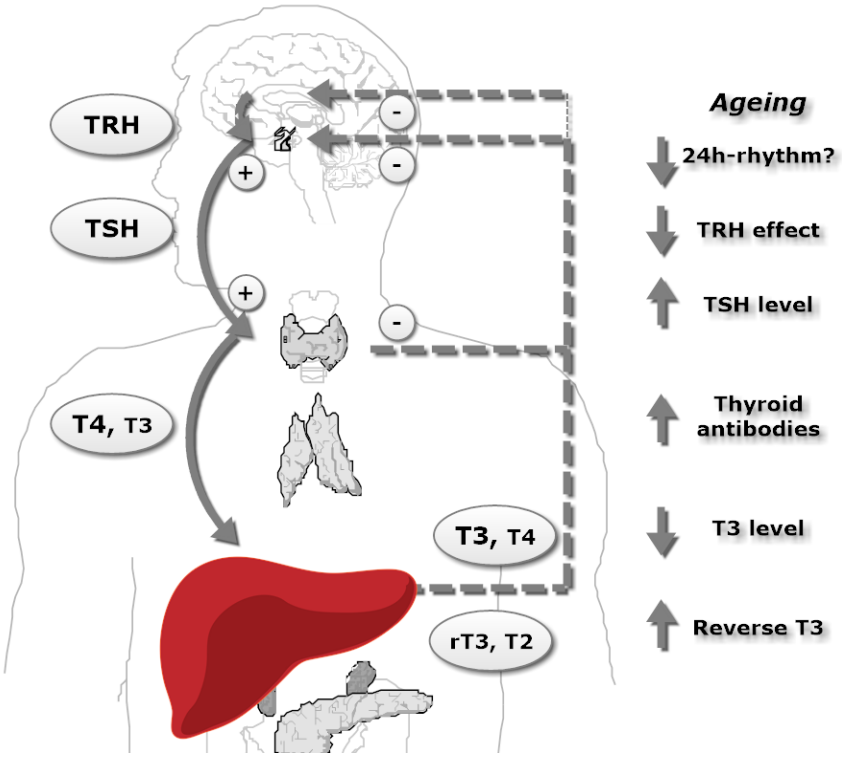

Figure 1.12. Human thyrotrophic axis and hormone secretion in ageing individuals. Adapted from *Veldhuis, 2013*.

Defining HPT axis status

TSH and Thyroid Hormones

HPT function is usually evaluated by plasma concentrations of TSH and TH. TSH serum half-life is around 50 minutes and, in the absence of hypothalamic-pituitary dysfunction, is the most sensitive parameter for evaluating a stable HPT status (D. Sarapura and H. Samuel 2017). The plasma concentration relationship between TSH and TH is almost logarithmic, where subtle changes of TH concentration will result into an amplified variation of TSH and a rapid return to a specific HPT homeostatic set-point (Hadlow, Rothacker et al. 2013). Although the population normal range for TSH plasma levels is wide, individual TSH levels are tightly controlled around a unique narrow interval. It is not known what defines this individual set-point but a follow-up study in healthy euthyroid subjects with repeated sampling have revealed a somewhat slim annual range of 0.75 mIU/l around a specific set-point, far less than the inter-individual variation within "normal" population reference levels (between 0.3 and 4.0 to 5.0 mIU/l) (Andersen, Pedersen et al. 2002, D. Sarapura and H. Samuel 2017).

Plasma concentration of T_4 and T_3 is interlocked to TSH levels and should always be interpreted in relation to it. With a clearance rate of about 7 days for T_4 and 24h for T_3, they allow a more prolonged and stable evaluation of the HPT set-point (Chatzitomaris, Hoermann et al. 2017, Molina 2018). As stated before, despite some direct non-genomic pathways, T_4 is considered mainly a "pro-hormone" that needs to be transformed into T_3. Therefore, when evaluating T_4 plasma levels it should be considered that they represent underlying thyroid TH production, whereas T_3 plasma levels are essentially looking at 24h liver and muscle tissue deiodination processes (Pilo, Iervasi et al. 1990). Because of their higher biological relevance and current good technical performance, FT_4 measurements by sandwich immunoassays have replaced total (free plus bound) T_4 determination. This methodology is also used for assessing FT_3 concentration but since this technique is not as validated as for FT_4, many authors state that determination of total T_3 (TT_3) should be favoured (Esfandiari and Papaleontiou 2017). A relative short intra-individual variation of FT_4 and TT_3 is also present in healthy euthyroid subjects with an intra-individual 95% confidence intervals range of around half that of the inter-individual group (Andersen, Pedersen et al. 2002). Still, free TH values measured by immunological methods are no longer valid in the presence of variations in the concentration of its carrier proteins. In such circumstances, the percentage of free hormone should be determined.

Despite its non-linear relationship, TSH–FT_4 ratio is an easy construct that allow a broader estimation of individual HPT status. Important caveats may arise when evaluating this measure in cohort studies, especially at the population level, where within physiological FT_4 concentrations in some ethnic groups and older people may present higher TSH levels (Surks and Boucai 2010). Apart from a very distinctive HPT set-point during the perinatal period, it is not known if there is a unique individual homeostatic TSH-FT_4 set-point that is preserved in a stable form during the life course. A hereditary effect has been proposed to account for 40-60% of the HPT set-point in each individual (Medici, Visser et al. 2015). However, data seem to indicate that there is a less constantly fixed set-point and suggest a more flexible balance point which result in a more adaptive allostatic HPT system (Chatzitomaris, Hoermann et al. 2017). An important issue to retain is that in almost all human cohort studies evaluating thyroid function and cognition, there was only one determination of TSH and FT_4 at baseline, per subject. This simplistic approach may be misleading, since it is currently known that for an accurate determination of each individual homeostatic set point (with a precision of around 10%) it is necessary to increase FT_4 and TSH testing up to at least five and twenty-five different samples, respectively (Andersen, Pedersen et al. 2002). For feasibility purposes it is often very difficult to obtain such many individual samplings in large cohort studies, but nevertheless it should be noted as an important limitation for most currently published studies.

Thyroid Volume and Morphology

Thyroid gland is one of the largest endocrine glands in humans. Several factors regulate its volume and internal structure, such as iodine delivery, age, gender and pregnancy (Dumont, Maenhaut et al. 1991, Vannucchi, Covelli et al. 2017). Although not entirely understood, it seems that most modulators act through local TSH-receptor follicular activation. In response to an insufficient supply of dietary iodine, some important modifications in thyroid structure and activity are generated by an increased secretion of TSH that follows a putative decrease in overall TH production. Prolonged iodine deficiency during thyroid development (and throughout life) is thought to be a major determinant for adult thyroid volume (WHO, UNICEF et al. 2007). Currently, one of the methods used to predict iodine deficiency status in the population is to determine the prevalence of goiter (increased thyroid volume) (Zimmermann and Andersson 2012). Standard WHO proposed "normal" adult thyroid volumes are to be of 7.7–25 ml in men and 4.4–18 ml in women but, due to many different confounders present in distinct populations, some national/regional reference

volumes have been proposed (Maravall, Gomez-Arnaiz et al. 2004, WHO, UNICEF et al. 2007, Kharchenko 2010). Synthesis of TH produces large amounts of H_2O_2 in the thyroid gland. Dietary iodine is rapidly oxidized by thyroid peroxidase in the presence of H_2O_2 and incorporated into tyrosine residues of thyroglobulin generating mono- and diiodotyrosine- molecules, the base blocks of all TH (Molina 2018). If this reaction is not correctly regulated by the anti-oxidation system, an imbalance may occur with concurrent tissue oxidation damage. This manifestation is thought to be the primordial pathogenic event linked to thyroid morphological and functional changes that occurs during thyroid autoimmune diseases and ageing (see *HPT axis and ageing*) (Burek and Rose 2008, Vitale, Salvioli et al. 2013). As a consequence, a typical finding will emerge on thyroid imaging with the emergence of diffuse heterogeneity and decrease echodensity (Kharchenko 2010). It could be speculated that adult thyroid volume and internal structure should be a surrogate marker of current and previous thyroid function "stressful" events, such as oxidative damage incidents and/or iodine deficiency.

Iodine Status

Urinary iodine excretion is an excellent indicator of recent iodine intake. This is based on the fact that dietary iodine is well absorbed through the digestive mucosa (over 90% of dietary intake) and that, in healthy adults, about 90% of it is excreted in urine within 24–48h (Nath, Moinier et al. 1992, Jahreis, Hausmann et al. 2001). Urinary iodine excretion can be determined by 24-hour urinary excretion (mcg/day), spot urinary iodine concentration (mcg/l) or morning spot urinary iodine to creatinine excretion ratio (mcg iodine/g creatinine). Twenty-four hour urine collections are more accurate for estimating individual iodine intake but are not feasible in large populational studies. Due to individual discrepancy on iodine intake, spot urinary iodine concentration presents such a large non-Gaussian daily variation that it is largely used for evaluating population iodine deficiency status (with a minimum sample of 50–100 individuals) and not for individual assessment of iodine status (Zimmermann and Andersson 2012). For epidemiological studies, WHO, UNICEF and Iodine Global Network (former International Council for Control of Iodine Deficiency Disorders) proposes 6 levels of iodine status based on the median spot urinary iodine concentration obtained in school-aged children: below 20 µg/(severe deficiency), between 20-49 µg/l (moderate deficiency), between 50-99 µ/l (mild deficiency), between 100-199 µ/l (adequate), between 200-299 µ/l (more than required) and above 300 µ/l (excessive) (WHO, UNICEF et al.

2007). Based on the proper interpretation of restricted adult population data, and despite the possibility of using these same cut-points when assessing participants over 18 years-old, many authors recommend less restrictive values with median cut-off of about 60–70 μ/l for "adequacy" (Zimmermann and Andersson 2012). Morning spot urinary relationship to creatinine excretion reduces variation introduced by distinct individual degrees of hydration but assumes a daily creatinine excretion rate that in lean or malnourished populations with poor protein intakes may be erroneous and generate even higher fluctuation. Therefore, if a large number of samples is obtained, median urinary iodine concentration in spot samples correlates better with the median from 24-h samples and avoid the need for extra urinary analyte determination (Konig, Andersson et al. 2011).

HPT axis and ageing

TSH and Thyroid Hormones

Ageing-related changes of the human HPT axis are currently not fully understood and subject to intense debate, mainly due to the influence of many aged-associated illnesses and chronic medication use on TSH secretion and deiodinase activities (Parle, Franklyn et al. 1991).

Epidemiological studies revealed that TSH plasma curves, in humans, are not normally distributed but rather skewed to higher concentrations with a shift tendency to even higher values in older healthy adults. Recent reanalysis of data coming from a large North America population-based group (n = 14,376) evaluated during the National Health and Nutrition Examinations Survey III (NHANES III), showed that in conditions of iodine sufficiency and absence of clinical or biochemical evidence of thyroid disease, serum TSH upper limit values increase from 3.5 mIU/l, in individuals younger than 50-years up to 7.5 mIU/l, for people older than 80-years (Surks and Hollowell 2007). This age shifting has been confirmed in different ethnic groups, such as Caucasians, Blacks and Hispanics, and also in cross-sectional studies coming from other populations in Europe and Australia (Surks and Hollowell 2007, Surks and Boucai 2010, Kahapola-Arachchige, Hadlow et al. 2012, Vadiveloo, Donnan et al. 2013). We have also observed similar findings in a Portuguese population-based sample (n = 972) with a mean TSH increase of 0.07 mIU/l per decade after 50 years of age (Carvalho, Correia Santos et al. 2014). Other studies with less restrictive exclusion criteria (*e.g.* accepting participants on thyroid substitutive therapy and with nodular goiter) described an inverse relationship with a negative age-dependent variations in serum TSH

concentration and mean levels (and upper limits) declining throughout older age groups (Mariotti, Barbesino et al. 1993, Booth, Deary et al. 2013). Fewer longitudinal studies have addressed TSH changes during ageing. Despite some methodological issues (*e.g.* different sensitivity on the TSH assays and exclusion criteria used during follow-up), most confirmed an upward shift of TSH levels during normal healthy ageing (Bremner, Feddema et al. 2012, Waring, Arnold et al. 2012, Mammen, McGready et al. 2017). Interestingly, in a 13-year follow-up cohort, despite an overall mean TSH increase of 0.32 mIU/l, the increment was most noticeable in elderly with a gender-adjusted TSH rise of 0.08 mIU/l for each decade over baseline age, similar to our cross-sectional study (Bremner, Feddema et al. 2012, Carvalho, Correia Santos et al. 2014). Despite these observations, individual TSH levels seems to be relatively stable throughout adult life. In a unique longitudinal study – the Baltimore Longitudinal Study of Aging, the authors observed that despite higher mean TSH values in older populations, most individuals presented a relatively stable thyroid function parameters with mean changes in TSH of around 0.006 mIU/l *per* year (Mammen, McGready et al. 2017). It was suggested that a survival bias could be present and that higher TSH levels observed in older population could simply be a consequence of a potential protective effect associated to individuals with higher TSH set point.

Observational human studies indicated that ageing is associated to an advance shifting of the pituitary (TSH) rhythm with no overall increase on 24-hour hormonal secretion (Ridgway, Weintraub et al. 1974, Roelfsema, Pijl et al. 2014). The relationship between TSH and TH also appears to change during normal ageing. Older subjects tend to exhibit an age-related increase in median TSH-FT_4 ratio, usually with FT_4 levels within reference range, but with relatively low T_3 levels (Jasim and Gharib 2018). Not all cross-sectional studies have observed this TSH-FT_4 tendency during ageing, with some describing an FT_4 upward curve during ageing whereas others observed no apparent difference between age groups (Mariotti, Barbesino et al. 1993, Hoogendoorn, Hermus et al. 2006, Carvalho, Correia Santos et al. 2014). In the few studies that evaluated FT_4 levels longitudinally, although no differences were found when comparing the age groups, a similar trend was present when considering each individual data over time (Bremner, Feddema et al. 2012, Waring, Arnold et al. 2012). In the Baltimore Longitudinal Study of Aging, FT_4 changes observed were less than 0.001 ng/dL per year (Mammen, McGready et al. 2017). In contrast to the conflicting data coming from TSH and FT_4 data, T_3 levels have been documented to consistently decline in elder populations, with some authors describing a reduction of up to 13% in total T_3 serum concentration over a 13-yr follow-up period (Mariotti, Barbesino et al. 1993, Waring, Arnold

et al. 2012, Carvalho, Correia Santos et al. 2014, Mammen, McGready et al. 2017). Accordingly, in a centenarian's cohort study, FT_3 was also found to be lower than in younger subjects, a trait that was curiously reported also in their offspring's when compared to age-matched controls (Corsonello, Montesanto et al. 2010, Garasto, Montesanto et al. 2017). The mechanisms mediating these findings are still not fully understood but may include a less robust feedback control of TSH secretion, changes in tissue deiodinases expression and a combination of chronic medication and age-associated illnesses that inhibit TSH secretion and alter deiodinase enzymes performances (Ridgway, Weintraub et al. 1974, Donda and Lemarchand-Beraud 1989, Over, Mannan et al. 2010, Veldhuis 2013). In a recent cross-sectional study evaluating more than 150,000 subjects, *Hadlow et al.* found that, within the same FT_4 range, older participants presented higher TSH levels than younger subjects, supporting the hypothesis that the age-related increase seen in TSH does not appear to occur due to any thyroid disease but rather as a consequence of age-related modification in HPT set point or reduced TSH bioactivity (Hadlow, Rothacker et al. 2013). Altogether, the current view supports that main age-associated modifications on the HPT axis (*i.e.* slight increase in TSH and T_3 decline) are potentially beneficial and not related to any negative ageing process (van den Beld, Kaufman et al. 2018).

Associated with these apparent HPT axis ageing features, other thyroid conditions are known to variate during senescence. Age-related changes involving the immune system can lead to an increase on autoimmunity diseases, especially in older female subjects (Gubbels Bupp, Potluri et al. 2018). Thyroid autoantibodies are circulating antibodies against several thyroid antigens [most commonly Thyroglobulin (Tg) and Thyroid-Peroxidase (TPO)] present in up to 27% of general population and in most patients with autoimmune thyroid disorders (Salvatore, Davies et al. 2011, Esfandiari and Papaleontiou 2017). Its prevalence is thought to be connected to genetic predisposition and environmental factors such as iodine insufficiency (Latrofa, Fiore et al. 2013). Iodine deficiency, potentiated by selenium deprival (deiodinases are selenoproteins) common in older individuals, is associated to thyroid vulnerability to oxidative stress and local H_2O_2 production needed for TH synthesis (Akbaraly, Hininger-Favier et al. 2007, Vitale, Salvioli et al. 2013). This increased exposure to oxidative stress may trigger a thyroid immune injury, which will ultimately lead to the appearance of thyroid autoantibodies (Burek and Rose 2008). Several epidemiological studies revealed that there is an overall increase of anti-Tg and anti-TPO antibodies in older individuals, which is more evident in women above 60-years, with no apparent increase in clinically overt thyroid autoimmune diseases (Pinchera, Mariotti et al. 1995, Hollowell, Staehling et al.

2002). Interestingly in several healthy elderly studies, serum thyroid autoimmunity was rarely found in centenarians when compared to younger individuals, suggesting a negative selection for individuals with evidence of autoimmune thyroiditis (Mariotti, Sansoni et al. 1992, Magri, Muzzoni et al. 2002). A possible connection between this auto-immunity phenotype and longevity have also been provided by genomic studies in animal models in which over-expression of immune genes, leading to immune hyperactivity and auto-immunity, was associated to shorter lifespan (Garschall and Flatt 2018). Yet in contrast, a recent population-based prospective study revealed that in older individuals (aged 85 or older), the presence of elevated anti-TPO antibodies levels over time were independently associated to a lower mortality risk with no significant changes in thyroid function or functional status (Du Puy, Poortvliet et al. 2019). These contradictory findings keep the question open over the role of thyroid autoimmunity in human ageing.

Thyroid Volume and Morphology

Substantial variation of thyroid gland morphology and structure is observed in adults even within the same age group. Most of this variation is modulated by gender, body-surface-area and iodine sufficiency status (Hegedus, Perrild et al. 1983, Barrere, Valeix et al. 2000). Thyroid volume has a positive correlation with body-surface-area and male gender; and a negative correlation with overall iodine intake. Despite some earlier studies, a general trend for thyroid gland atrophy with advancing age has been recognized by recent studies performed in iodine replete populations using various thyroid imaging techniques (Denham and Wills 1980, Mahne, El-Haddad et al. 2007). This trait may reflect the subjacent reduction in the average size of follicles that results from a decrease in colloid content and an expansion of interfollicular fibrosis, many times associated with chronic underlying autoimmunity processes (Blumenthal and Perlstein 1987).

Nodular goiter is a clinical concept that represents an enlarged thyroid by nodules. The presence of benign nodular thyroid lesions is highly prevalent during ageing, especially in iodine-insufficient regions where slow nodular growth will result in large multinodular glands. Clinically relevant nodules are found in approximately 5-15% of the population but, when reviewing thyroid imaging and autopsy based studies, its prevalence appears to be much higher, around 50% of 50-year old subjects and almost 100% of women by 90 years (Denham and Wills 1980, Hintze, Windeler et al. 1991, Dean and Gharib 2008). Thyroid nodules do not always coincide with a clear morphological definition and may include simultaneously colloid nodules or epithelial tumours (such as adenomas

and carcinomas) (Hedinger, Williams et al. 1989). With advancing age, and especially in iodine deprived settings, there will be enlargement of thyroid follicles and increase in follicular cysts and interstitial fibrous tissue, that will culminate into the gross nodularity found in older thyroid glands (Blumenthal and Perlstein 1987, Stan and Morris 2005). Genetic factors and intra-thyroidal cumulative oxidative stress damage (stimulated by iodine and selenium deficiencies) associated with increased levels of TSH, are thought to be involved in the pathogenesis of this condition (Paschke 2011). However, it is not known if this represents actually a "healthy" senescence process of an iodine deficient population or rather a pathologic late expression of thyroid dysfunction. In line with the later hypothesis, a rare report with thyroid ultrasound evaluation of centenarian subjects, involving a border-line iodine-sufficient Danish population, found that these individuals present smaller thyroid volumes, low prevalence of nodularity (around 25%) and no concomitant increase of thyroid dysfunction (Andersen-Ranberg, Jeune et al. 1999).

Recently, an interesting and comprehensive concept of "thyroid biography" has been proposed by *Franceschi et al.* in order to better incorporate the complex history behind thyroid status among the elderly (Franceschi, Ostan et al. 2019). In their proposal the authors envisage the need for lifelong collection of data on thyroid function and morphology with the aim of better characterizing life events and matching HPT axis adaptative responses, therefore helping clarify some of individual age-related variations.

HPT axis and cognition

Thyroid hormones are critical for brain development and performance throughout all human lifespan. Since the 19th century, it has been documented that normal thyroid function is essential for brain growth and mental functioning (Ord 1888, Doyle 1991, LaFranchi and Huang 2016). In humans, irreversible brain damage and gross mental retardation, along with varying degrees of short stature, deaf-mutism and motor spasticity (cretinism) was associated thyroid dysfunction due to severe *in utero* iodine insufficiency (DeLong, Stanbury et al. 1985, Chen and Hetzel 2010). Untreated congenital hypothyroidism, as a result of thyroid dysgenesis or inborn errors of thyroid hormone synthesis, was also connected to severe and irreversible neurological syndromes, stressing the essential role of TH during early stages of brain development (LaFranchi and Huang 2016). As an interesting note, already in the 1890's Portuguese physicians were among the first addressing this topic and started testing hypodermic transplants of sheep thyroid gland in severe

hypothyroid patients with some success in temporarily reversing serious neurological dysfunction (Bettencourt and Serrano 1891).

Despite all these anecdotal clinical examples and therapeutic proposals, it was only in the 1920's that successful therapeutically agents were finally offered to hypothyroid individuals when the first synthetic production of thyroxine was presented by *Harington and Barger*. After that, public-health policies addressing iodine insufficiency were consistently implemented (Harington and Barger 1927, Zimmermann and Andersson 2012). In the early 1950's researchers developed animal models to study the real effect of TH on brain maturation (Eayrs and Taylor 1951, Eayrs and Horn 1955). Within the following years it became clear that TH were not only a major player during the very early steps of brain development, such as neural induction, neurulation and establishment of polarity and segmentation, but also essential for many other later events, such as migration and differentiation of neural cells, synaptogenesis and myelination (Bernal 2007). Later studies found that TH action in the foetal brain also predated the onset of foetal thyroid gland growth, underlining the importance of maternal derived TH during early central nervous system development (Calvo, Obregon et al. 1990). Important scientific work focusing on cerebellar development showed a TH direct effect in foliation and laminar organization, Purkinje cells and granule cell differentiation and migration, as well as dendritic arbor elaboration (Nicholson and Altman 1972, Nicholson and Altman 1972). In further studies, some neocortical, hippocampus and glial cell maturation disturbances associated to pre- and peri-natal TH deficiency, were also recognized. This paved the proposal for the three stages of TH-dependent neurological development by *Porterfield and Hendrich* (Schapiro, Vukovich et al. 1973, Ruiz-Marcos, Sanchez-Toscano et al. 1979, Porterfield and Hendrich 1993, Berbel, Guadano-Ferraz et al. 1994). The first proposed stage (Phase 1) is a period before the onset of foetal pituitary-thyroid axis function (around 10 to 16 weeks of gestation in humans), when the developing foetal brain is exposed exclusively to maternally produced TH (Figure 1.13). During this phase, most of brainstem and cerebral neurogenesis and neuronal migration take place. The second stage (Phase 2) is the remaining *in utero* life period, where the source of TH is mostly dependent on active foetal thyroid function but still reliant on some maternal TH and, importantly, on continuous maternal iodine supply. Throughout this period, the human foetal brain is experiencing significant neuronal migration and maturation, axonal outgrowth, dendritic branching and synaptic development, as well as glial cell differentiation, migration and early myelination. The final proposed stage (Phase 3) occurs after birth and represents a period where TH is entirely produced by the neonate's thyroid and totally dependent on regular iodine

supply from the diet. During this phase most gliogenesis and myelination take place, with simultaneously forebrain neuronal maturation, dendritic branching, synaptic development and a continuing migration of granule cells in the hippocampus and cerebellum.

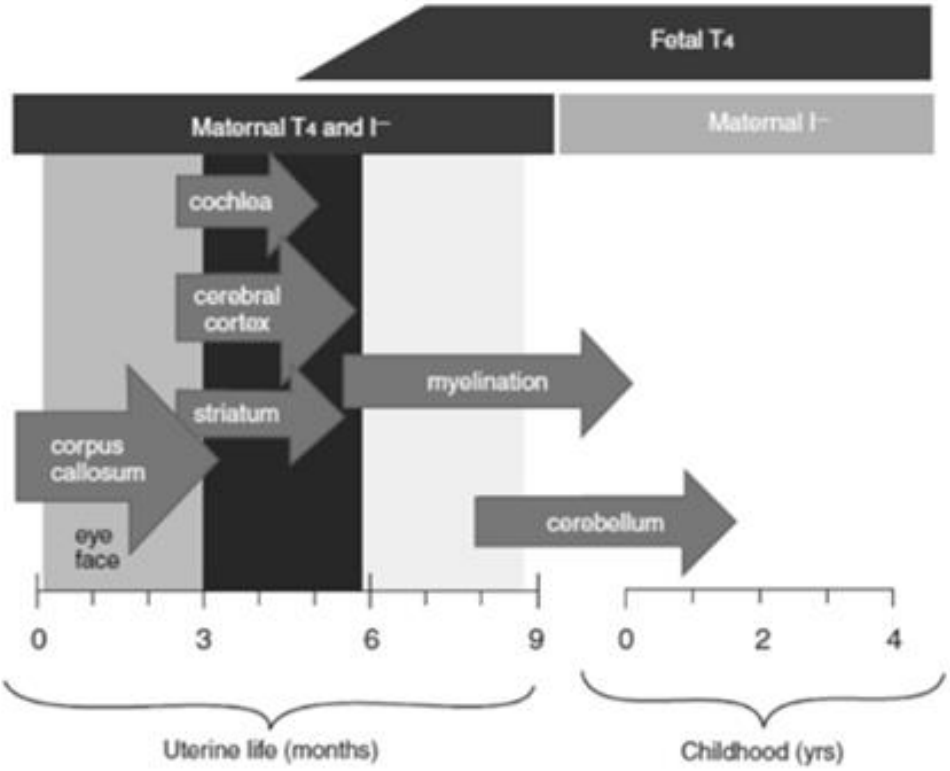

Figure 1.13. Timing of thyroid hormone action in the developing human brain. Adapted from *Zoeller, Rovet et al. 2004*. I-, iodine.

Experimental manipulation of TH levels during similar phases in animal models, and pre-natal/congenital TH deficiencies in observational studies in humans, have been essential for understanding the mechanisms behind thyroid influence during CNS stages of development. Thyroid function loss only during prenatal period predisposes to visual and visuomotor difficulties, whereas TH insufficiency during early neonatal is primarily associated to visuospatial impairment (de Escobar, Obregon et al. 2004). Deficiency of TH present only during childhood is linked to sensorimotor and language deficits and, further in infancy, to fine motor, auditory processing, attention, memory and executive processing impairment (Zoeller and Rovet 2004, Moog, Entringer et al. 2017).

Nuclear TH receptors mediate TH main actions in the brain. For its correct function their location and timing are essential. For example, morphological brain changes secondary to thyroid dysfunction might not be a consequence of low plasmatic TH levels but actually an effect of time-depend local altered transcriptional activity of unliganded TH receptors (Bernal 2007). As previously mentioned, the regulation of TH-dependent gene expression is influenced by 1) local ligand availability, especially by TH uptake through the blood brain barrier (BBB) and choroid plexus; 2) TH intra-cellular metabolism and transport; 3) expression and distribution of the TH receptors isoforms; 4) balance of TH nuclear receptor corepressors and coactivators; and 5) genome location of TH nuclear response elements (TRE).

Impairment of TH uptake by the BBB present in brain blood capillaries and choroid plexus, due to disturbances in the transporters and receptors system, such as LAT1, LAT2, OATP1C1, MCT8 and MCT10, have been associated to cerebellar development delay, impaired myelination and poor motor performance in animal models (Faustino and Ortiga-Carvalho 2014, Mayerl, Muller et al. 2014). This was partially corroborated when individuals with a clinical condition mimicking congenital hypothyroidism but with relatively "benign" blood TH hormone concentrations (Allan-Herndon-Dudley syndrome), were found to have a loss-of-function mutation of the X-linked MCT8 gene (or solute carrier family 16 member 2 - *SLC16A2*) (Schwartz, May et al. 2005, Ramos 2014).

After T_4 uptake by BBB and glia, brain T_3 is primarily generated inside astrocytes through deiodination by type 2 deiodinase. T_3 is then transported into neurons through MCT8 and finally presented to TH nuclear receptors or inactivated by type 3 deiodinase (Friesema, Ganguly et al. 2003, Dentice, Marsili et al. 2013). These TH transporters and receptors are thought to be expressed in the brain in a somewhat specific temporal and spatial pattern. For example, MCT8 transporter is highly expressed very early in the hypothalamus, TRα1 has been localized in all CNS regions throughout life, whereas TRβ1 and TRβ2 have been found in more restricted areas such as the hypothalamus, pituitary and retina, especially during later stages of development (Bernal 2007, Flamant, Gauthier et al. 2017, Anyetei-Anum, Roggero et al. 2018).

Adult brain morphology and function

Early studies in 1950's have found that in hypothyroid rats, overall brain volume was smaller than those of controls (Eayrs and Horn 1955). Later work has described specific brain areas that are more susceptible to thyroid function. In rodent models, TH insufficiency during brain development

is associated to reduced pyramidal cell layer (CA1 and CA3) and nerve fiber numbers in the hippocampus (Madeira, Sousa et al. 1992, Gong, Dong et al. 2010). In adult mammals, neurogenesis present in subventricular and subgranular zones of the dentate gyrus is also influenced by TH presence with hypothyroidism preventing normal subventricular zone progenitor cell-cycle re-entry and migration, an effect that can be rescue by T_3 administration (Lemkine, Raj et al. 2005). Other chief brain areas affected by even modest reductions of gestational TH levels are the neocortex and corpus callosum. Cortical heterotopias (see above *Brain development*) are frequently found in animals with pre-natal TH insufficiency. This feature persists during adult age, even if a return to normal thyroid status is obtained, and has important brain functional implications with increased risk of seizures (Hannan, Servotte et al. 1999, Auso, Lavado-Autric et al. 2004, Goodman and Gilbert 2007).

Human imaging studies

In human, imaging studies have shown a reduction of hippocampal volume in children with congenital hypothyroidism but also in those born from mothers with hypothyroidism (affecting particularly the left hippocampus) and in adult-onset hypothyroidism individuals (Wheeler, Willoughby et al. 2011, Cooke, Mullally et al. 2014, Willoughby, McAndrews et al. 2014). In a large Dutch prospective cohort study, maternal hypothyroxinemia (low T_4 levels in the presence of "normal" TSH levels) during early pregnancy did not change total brain volume and cortical thickness in the offspring's when evaluated at 6-years old (Ghassabian, El Marroun et al. 2014). More recently, the same cohort was evaluated after a further 3 to 6 years follow-up extension and found that previously "low" and "high" maternal TSH concentration groups, between 8 and 13 weeks of gestation, were associated with a smaller volume of total grey matter and cortical grey matter in children aged 9–12 years (Jansen, Korevaar et al. 2019). Interestingly, T_4 levels did not predict brain morphology changes. These results support the idea of a non-linear TSH effect on total grey matter or cortical grey matter development in the pre-adolescent brain as well as the existence of a critical early gestational time window for maternal thyroid dysfunction.

In adults, TSH levels have been inversely associated to hippocampal (bilaterally) and white-matter volume in a cross-sectional population-based study (Ittermann, Wittfeld et al. 2018). However, no association was found with FT_3 and FT_4 levels, suggesting that overt hypothyroidism is not a necessary condition to reduce total brain or hippocampal volume. In a publication from the

Rotterdam Study cohort evaluating 4,683 euthyroid adults (mean age 60-yr), TSH serum concentration was found inversely connected to overall brain volume (Chaker, Cremers et al. 2018). Higher FT_4 levels related to larger total brain and white-matter volumes in younger persons; on the contrary, in older individuals it was associated to smaller total brain volume – mostly due to a larger reduction on white-matter. Importantly, these findings on FT_4 and TSH with total brain volume across different age groups were almost exclusively attributed to changes in white-matter volume. Adjusting for cardiovascular risk factors did not change these observations, suggesting that, at least in this cohort, TH-white-matter volume link is probably independent of any brain vessel compromise.

In adult brains, TH levels are apparently not related to overall grey-matter volume (Chaker, Cremers et al. 2018). Some authors tried to recognise potential associations between TH and specific brain regions using voxel-based morphometry imaging techniques. In a recent study exploring this method in healthy adult individuals, FT_4 levels were positively associated to an area in the left middle frontal gyrus (Brodmann area 9), a region linked to the hippocampus and to short term memory performance (Babiloni, Ferretti et al. 2005, Ittermann, Wittfeld et al. 2018). In other two small studies evaluating hyperthyroid patients, TH were linked to lower volumes of the left hippocampus, para-hippocampal gyri (bilateral), visual cortex and anterior cerebellum; and inversely to an increase of grey-matter volume in the right posterior cerebellum (Zhang, Song et al. 2014, Gobel, Heldmann et al. 2015).

The role of TH on human brain function has also been explored through PET-FDG and fMRI studies. The first published works evaluating severe hypothyroid patients found a generalized decrease in cerebral glucose metabolism and blood flow, with no apparent regional distinction (O'Brien and Harris 1968, Constant, de Volder et al. 2001). More recently, arterial spin labelling MRI studies indicates that, during hypothyroid state, cerebral blood flow is significantly decreased in some brain posterior parts (especially in cerebellum, left fusiform gyrus, left thalamus, and left red nucleus) and increased in the frontal gyri (Kaichi, Kenjo et al. 2015). Other fMRI studies detecting haemoglobin oxygenation intrinsic changes (Blood-oxygen-level-dependent – BOLD responses) showed that in mild hypothyroid individuals, multiple brain areas linked to memory and attention performance, such as subgenual and perigenual anterior cingulate cortex, posterior cingulate cortex, amygdala and hippocampus, are also affected with a diminished activity when compared to controls (Bauer, Silverman et al. 2009). Working memory processing in mild hypothyroidism, revealed a reversible task-induced deactivation of some other brain regions considered to integrate

the default mode network, such as the medial prefrontal and posterior cingulate cortices, and the left inferior partial lobule (He, Ma et al. 2011). In this specific memory task, individuals with subclinical hypothyroidism (defined by an elevation of TSH in the presence of normal free circulating TH) executing verbal working memory tests also presented altered brain activities in both bilateral frontal areas (Zhu, Wang et al. 2006, Yin, Liao et al. 2013). Comparing to healthy controls, clinical and subclinical hypothyroids displayed decreased functional connectivity of the right frontoparietal network (frontal pole), medial visual network (lateral occipital gyrus, precuneus cortex and cuneus) and motor network (precentral gyrus, postcentral gyrus, precuneus cortex, paracingulate gyrus, cingulate gyrus and supramarginal gyrus) (Zhu, Wang et al. 2006, Singh, Kumar et al. 2015, Gobel, Gottlich et al. 2019). In agreement with many other follow-up studies, none of these findings was maintained when stable euthyroidism was restored (Quinque, Karger et al. 2014). Long lasting effects of TH insufficiency over human brain function are difficult to obtain and have only been explored assessing cohorts of individuals with congenital hypothyroidism. A Canadian cohort of young adolescents diagnosed with congenital hypothyroidism who were first treated within the first two weeks of life, described that during visuospatial associative memory tasks, there was an increased activation of the left hippocampus proportional to the severity of TH deficiency at diagnosis (Wheeler, McAndrews et al. 2012, Wheeler, McLelland et al. 2015). But when tested for verbal associative memory tasks, which require primarily the left hippocampus, there was no difference to controls. These results may indicate a potential early permanent adjustment of functional integrity of right hippocampus and the necessity for additional neural resources (*i.e.* left hippocampus) in order to complete visuospatial memory tasks in patients with history of congenital hypothyroidism.

Few human brain imaging studies have been published exploring the reverse spectrum of thyroid function – thyrotoxicosis. Human brain exposed to excess TH serum levels have been shown to reduce glucose metabolism of the frontal, temporal and limbic areas (Schreckenberger, Egle et al. 2006, Miao, Zhang et al. 2011). Glutamate concentration is decreased in posterior cingulate cortex of these patients, strengthening the idea of potential TH-dependent glutamate clearance mechanisms (Mendes-de-Aguiar, Alchini et al. 2008, Liu, Bai et al. 2012). During short-lived thyrotoxicosis status, individuals performing working memory tasks usually present normal functioning of frontal and parietal areas with an increase in resting-state functional connectivity in rostral temporal lobes, suggesting an amplified functional connectivity and prefrontal control over limbic areas (Zhu, Wang et al. 2006, Gottlich, Heldmann et al. 2015). On the other hand, when

evaluating chronic hyperthyroid patients with autoimmune Graves' disease, a decrease was found in the intrinsic functional connectivity of cerebellum left posterior lobe and medial frontal gyrus, with concomitant abnormal low resting-state functional connectivity inside default mode, attention, visual and cognitive networks (Li, Zhi et al. 2017). Whether these variances between the two distinct thyrotoxicosis models are due to the chronic exposure to high levels of TH or to an underlying autoimmune basis of Graves' disease, remains to be clarified. Just recently, another study evaluating brain resting-state connectivity in new diagnosed thyrotoxicosis patients reported similar findings with decreased intra network functional connectivity of fronto-temporal network, default mode network, fronto-parietal network, also in sensorimotor network and salience network regions, reinforcing the importance of TH on visuospatial and executive processing in these individuals (Kumar, Rana et al. 2019). In a single study evaluating resting-state functional connectivity pattern of hippocampus in untreated Graves' disease patients, hyperthyroidism was found to weaken hippocampus pathways to bilateral anterior and posterior cingulate cortex, which could explain some of the alterations found in mood and memory processing observed in thyrotoxic patients (Zhang, Liu et al. 2014).

Very few studies explored the relationship between TH levels and functional brain imaging in healthy euthyroid individuals. Preliminary work from our team examined the association between HPT axis and brain resting state networks in a healthy aging cohort (Fernandes 2015). In this exploratory study with 87 participants (aged 51 to 82 years, 55% male) it was found that TSH levels present a positive correlation with dorsal mode network activity in males, but not in females, and a negative association with dorsal attention and insular-temporal networks only in females. When analysing FT_4 levels, only ventral attention network activity was associated with gender in an asymmetrical manner. Left ventral attention network (particularly the Brodmann area 6 and 10) was positively correlated to FT_4 serum levels in males, but negatively in females. Right ventral attention network was only positively correlated in females. Additionally, in males, total T_3 serum levels were positively correlated to functional connectivity of regions of the insular-temporal network relevant for short term memory and auditory verbal attention, such as Brodmann area 9. By contrary, in these same subjects, T_3 was negatively associated to activity of primary visual and right ventral attention networks. Again, in females, a distinct pattern was observed with T_3 serum levels negatively correlated to sensorio-motor network activity and in particular the Rolandic operculum. The implications of these apparent idiosyncratic gender effects of HPT status on brain function during healthy aging is not currently understood.

Published data addressing HPT axis hormones and their relationship to brain functional connectivity are scarce. In general, among overtly hypothyroid patients, a negative correlation was found between TSH and FT_4 levels with cuneus connectivity, and left amygdala to right middle temporal gyrus networks, respectively (Quinque, Karger et al. 2014, Li, Zhi et al. 2017, Gobel, Gottlich et al. 2019). Serum FT_3 levels were only described, in untreated hyperthyroid patients, as negatively correlated to functional connectivity between right posterior cingulate cortex and left anterior insula (Liu, Ran et al. 2017).

Human Cognitive studies

Previously to first studies reporting extensive effects of TH on the overall brain development and neuronal function, many others have demonstrated that TH deficiency in humans can lead to important cognitive consequences. Since the 1960's, several authors proposed that the presence of relative or absolute TH deficiency during pregnancy, mainly due to iodine deficiency or maternal hypothyroidism, was associated to offspring mental retardation, learning disabilities and motor abnormalities (Choufoer, Vanrhijn et al. 1965, Man and Jones 1969, Pharoah, Buttfield et al. 1971, Pharoah, Connolly et al. 1984). Some later reports described that these features were correlated only to low maternal serum T_4 levels and not to any maternal serum T_3 or TSH changes, implying that even "subtle" variations in thyroid function during early stages of brain development would carry significant future cognitive consequences (Pop, Kuijpens et al. 1999, Morreale de Escobar, Obregon et al. 2000, Costeira, Oliveira et al. 2011). After several observational and intervention studies, it is currently consensual that iodine repletion in severe to moderate deficient population has a significant impact in current and later generations cognitive performance (Zimmermann, Connolly et al. 2006, Zimmermann and Boelaert 2015).

Overt hypothyroidism studies

In adults, overt hypothyroidism has been classically considered as a treatable cause of cognitive impairment (Cummings and Benson 1983). In non-demented older adults, hypothyroidism is associated to apparent reversible impairment in learning, word fluency, visual-spatial abilities, and some aspects of attention (Osterweil, Syndulko et al. 1992, Constant, Adam et al. 2005). Yet this statement has been recently challenged when some population-based studies failed to find such a connection, especially when more comprehensive cognitive domains were tested and individuals

with criteria for dementia were excluded (Kim, Stewart et al. 2010, Parsaik, Singh et al. 2014). In an Italian population-based cohort followed for 4-years (that didn't exclude overt hypothyroid participants), baseline TSH was related to an increased risk of vascular dementia, but not of developing mild cognitive impairment or Alzheimer dementia (Forti, Olivelli et al. 2012). Interestingly, despite a somewhat negative TH regulation of β-amyloid precursor protein expression, human brain studies have failed to show any association between TSH levels and cerebral β-amyloid protein deposition or neurodegeneration (Belandia, Latasa et al. 1998, Choi, Byun et al. 2017). More evidence for the lack of connection between high TSH levels and dementia is also provided by cohorts in Northen Europe and USA, some of which included autopsy sub-studies. In these reports, baseline higher serum TSH concentration was not related to latter incidence of Alzheimer dementia or any Alzheimer-type neuropathology features (Kalmijn, Mehta et al. 2000, de Jong, Masaki et al. 2009).

Subclinical hypothyroidism studies

Since thyroid dysfunction can be seen as a *continuum* and because subclinical hypothyroidism is also linked to a cardiovascular risk increase, including stroke events, it is plausible that these individuals could be predisposed to premature mild cognitive impairment and vascular dementia (Chaker, Baumgartner et al. 2015). There are conflicting data when evaluating adult subjects with this subclinical dysfunction. In some cross-sectional studies, increased impairment of verbal and visual memory, and reduced MMSE performance were described (Monzani, Del Guerra et al. 1993, Baldini, Vita et al. 1997, Cook, Nebes et al. 2002, Hogervorst, Huppert et al. 2008, Resta, Triggiani et al. 2012). On the contrary, more recent cross-sectional and longitudinal studies with improved methodologies (*e.g.* larger samples controlled for mood alterations and performing more exhaustive neurocognitive tests) failed to find any significant cognitive effects of subclinical hypothyroidism, at baseline or during follow-up. In a large population-based study with near 6,000 subjects over 65-year old, no differences were found between subjects with subclinical hypothyroidism (n = 168) and euthyroid subjects, when explored by MMSE and Middlesex Elderly Assessment of Mental State batteries (Roberts, Pattison et al. 2006). However, these tests were designed to detect gross global cognitive impairment in elderly subjects and might not detect subtle deficits induced by such a mild thyroid dysfunction. In another large longitudinal study in 5,100 men and women with a high vascular risk (aged 70–82 years), subclinical hypothyroid participants (n = 161) did not present a

worse performance than euthyroid controls at neuropsychological tests used to measure executive function and memory, both at baseline and after 3-years follow-up (Wijsman, de Craen et al. 2013).

In order to review all available data a meta-analysis was published in 2015 which included 9 cross-sectional and 6 longitudinal studies, incorporating more than 1,000 community-dwelling elderly participants with subclinical hypothyroidism (Akintola, Jansen et al. 2015). Despite significant heterogeneity for TSH cut-point values and for cognitive domains used, the authors did not find evidence supporting the association between subclinical hypothyroidism and cognitive impairment, namely over global cognition, memory and executive function domains. Another recent meta-analysis evaluated 13 studies (4 cross-sectional, 8 prospective and one case-control) for associations between subclinical hypothyroidism and a composite "cognitive" endpoint of incidence/prevalence of dementia or decline on MMSE and Wechsler Memory and Adult Intelligence scores (Pasqualetti, Pagano et al. 2015). Consistent with the previous meta-analysis, this report also failed to observe relationships between overall mild thyroid failure and cognitive decline or dementia. However, when restricting participants to include only individuals younger than 75-years, subclinical hypothyroidism was associated to an increased risk of dementia and poorer cognitive performance. Regardless of some potentially publication bias, this finding can also be explained by an overdiagnosis of subclinical hypothyroidism present in some of the elderly cohorts' studies included. The lack of consensus for age-related TSH reference ranges can result in selection of some borderline "euthyroid" older participants to the "subclinical hypothyroidism" group, therefore reducing the risk of cognitive decline observed in this cluster when compared to "true" subclinical hypothyroid elderly individuals.

Table 1.9. Key points about thyroid function and brain performance.

- TH deficiency during brain development has a profound effect on latter overall cognitive performance
- Both low and high levels of TH are inversely related to hippocampal and white-matter volume in adults
- Overt hypothyroidism is associated to reversible impairment of memory and attention
- Excess circulating thyroid hormones may result in neuronal loss, diminished brain connectivity and declined global and executive cognitive functions
- Reverse causality and excessive heterogeneity among human studies weaken any definite conclusions

Thyrotoxicosis studies

Recently diagnosed thyrotoxicosis patients have been shown to perform worst in MMSE and some executive functions, with significantly decreased functional connectivity in several relevant neural networks (such as frontotemporal, default mode and salience networks), with no deviations in memory and attention domains (Vogel, Elberling et al. 2007, Kumar, Rana et al. 2019). Despite this observation, very few human studies have addressed the effect of chronic overt hyperthyroidism status over cognitive function and the risk of dementia, probably due to ethical issues. In a small cohort of patients on suppressive treatment with levothyroxine (with serum TSH below detection levels) for thyroid cancer carcinoma, it was found that executive functions and working memory were negatively affected by the thyrotoxicosis status (Jaracz, Kucharska et al. 2012).

Subclinical hyperthyroidism studies

More frequently, subjects with *subclinical* hyperthyroidism (*i.e.* individuals with subnormal TSH levels but with T_4 serum concentrations within reference ranges) have been available in order to search for thyroid function connection with cognitive impairment and dementia. Within this spectrum of dysfunction, epidemiological studies provided inconsistent data. Several cohorts reported that subclinical hyperthyroidism was associated to an increased risk of dementia and worse global cognitive performance. In a cross-sectional population-based Brazilian study, with more than 1,200 participants over 65-years, a positive association between subclinical hyperthyroidism and dementia was found, specifically with vascular dementia (Bensenor, Lotufo et al. 2010). Similarly, in a large Italian population-based study with more than 900 individuals aged 65-year or plus, MMSE scores were found to be lower among those with subclinical hyperthyroidism when compared to the euthyroid group (Ceresini, Lauretani et al. 2009). On the other hand, *Roberts et al.* in the largest ever published cross-sectional population-based study evaluating cognition and subclinical thyroid dysfunction with nearly 5,900 participants, found no connection between subclinical hyperthyroidism and cognitive performance, despite a small relationship between higher serum concentrations of FT_4 and MMSE performance (1-point increase in the MMSE score for each increase of 25 pmol/l of FT_4 serum concentration – normal range from 9.0 to 20.0 pmol/l), (Roberts, Pattison et al. 2006).

Prospective longitudinal analyses provided more consistent evidence that subclinical hyperthyroidism is indeed associated to several downward cognitive domain trajectories. In the Rotterdam Study, during a 2-year follow-up, a 3.5 increased risk of dementia from all causes was found among those with a subnormal TSH levels at baseline (Kalmijn, Mehta et al. 2000). In a more recent study with a large Scottish population-based cohort involving more than 12,000 participants with a mean age of 66.5 years (and a median follow-up period of over 5 years), subclinical hyperthyroidism was also associated to an 1.8 increased risk of dementia (Vadiveloo, Donnan et al. 2011). It is worth noting that in this study only subjects with two baseline confirmatory measurements of TSH (at least 4 months apart) were included and that all those who developed overt thyroid disease during follow-up were further excluded. In contrast, a Dutch longitudinal study (Rotterdam Scan study) involving 1,025 individuals aged 60 to 90 years, did not find an association between lower TSH levels and incidence of dementia during their mean 5.5 years follow-up (de Jong, den Heijer et al. 2006). Similarly, another prospective longitudinal study of 5,154 participants aged 70–82 years with high cardiovascular risk (PROSPER study) also failed to observe any link between subclinical hyperthyroidism and cognitive decline or impairment during old age (Wijsman, de Craen et al. 2013). Despite these inconsistencies, published evidence supports that subtle thyroid hyperfunction has a negative impact on cognitive performance over time. A meta-analysis published in 2016 evaluated five prospective cohorts, which included more than 200 participants with subclinical hyperthyroidism, recognised that pooled adjusted risk ratio for dementia was 1.67 in these subjects (Rieben, Segna et al. 2016). Nevertheless, their mean MMSE decline from baseline until the end of follow-up (mean 32 months) was not found to differ from controls.

Euthyroidism studies

Despite some incongruent findings when evaluating thyroid dysfunction at the extremes of normality, observational studies provided some evidence linking thyroid function and cognitive performance when evaluating only euthyroid individuals. In one of the first cross-sectional studies, 72 healthy men were examined with a series of cognitive tests assessing memory, performance intelligence, verbal abilities and reaction time speed, authors reported that only total T_4 was positively associated to any measure of overall cognition (Prinz, Scanlan et al. 1999). In a sample of 120 euthyroid participants recruited from the Maastricht Aging Study, aged between 49 and 71 years, no interaction between TSH and cognition was found (no data on T_4 was provided) (van

Boxtel, Menheere et al. 2004). In a more recent cross-sectional study with more than 1,200 healthy elderly participants, higher FT_4 levels (within normal reference range) were associated to better performance on visuo-spatial/visuo-construction ability and psychomotor speed (Beydoun, Beydoun et al. 2013).

The Women's Health and Aging Study enrolled 464 euthyroid women (aged 65-years or more) and reported no association between baseline TSH or T_4 levels and cognitive function measured by MMSE test, but after 3-year follow-up, lower total T_4 levels (within reference range) were associated with a greater risk of cognitive decline (Volpato, Guralnik et al. 2002). By contrast, in a longitudinal study with 1,047 older euthyroid adults followed for 2 years *Hogervorst et al.* reported that high normal baseline FT_4 levels were associated with accelerated cognitive decline. In favour of this U-shape relationship between "normal" T_4 levels and cognitive performance, *Yeap et al.* found that, in 3,401 older men, higher FT_4 levels predicted new-onset dementia with an 11% increase in risk per 1 pmol/l increment in FT_4 (if maintained within reference range) with no relationship between TSH and risk of dementia during a mean follow up of 5.9 years (Yeap, Alfonso et al. 2012). More recently, in a large longitudinal study of USA community-dwelling older adults (n = 2,843) with a follow-up period of 17 years, the authors reported a lower incidence of dementia within participants with "normal" TSH concentrations in the fourth quartile, even after adjustment to multiple covariates, but not in those with lower FT_4 levels (Cappola, Arnold et al. 2015). In a Swedish study involving a longitudinal assessment of both thyroid indicators and cognition, *Wahlin et al.* showed that, euthyroid population aged 75 years and older with declining TSH levels (within reference range) were associated with declining verbal fluency and visuospatial abilities (Wahlin, Bunce et al. 2005). At six-year assessment, episodic memory was also positively related to TSH levels changes, but when evaluating its longitudinal performance, no TH-associated cognitive decline trend was observed.

Findings from studies in younger euthyroid cohorts are also controversial. When analysing the NHANES III cohort separately by age categories and excluding out of range TH levels, FT_4 and TSH concentrations were associated with distinct performances over different episodic and working memory tests (Beydoun, Beydoun et al. 2012). In the 20–59-year-old group, FT_4 was linked to poorer performance in serial digits learning test, while in the 60–90-year-old group it was related to a better performance in serial 3's subtraction task. On the other hand, higher TSH was related to worse performance on symbol digits substitution test-latencies in the 20-59-year-old group while in the 60–older group was rather linked to improved performance in story recall and serial 3's

subtraction test. This inverse TSH relationship with cognitive outcomes in younger subjects was also found in a cross-sectional study which included 9,769 middle-age participants (mean age of 49.5-years) with TSH levels within reference range. In this population the first TSH tercile group (below 1.20 mIU/l) was associated to worse performance on some executive function tasks (trail making test), even when adjusted to multiple demographic, social and clinical variables, with no effect on verbal fluency or episodic memory tests (Szlejf, Suemoto et al. 2018).

There is no current explanation for these somewhat contradictory findings between TH and different cognition domains in distinct age groups. Despite extensive adjustments done by many authors, some of the results may still be affected by residual confounding. In order to clarify some of these results, future studies must address the potential effect of TH on cognitive performance during diverse time-windows of brain development and stages of normal ageing.

In a 2016 meta-analysis that included 24,952 euthyroid participants from three case-control and eight cohort studies, higher levels of FT_4 were associated to dementia (RR = 1.08, 95% CI 1.00–1.17; for each FT_4 standard deviation increase), but TSH levels within the lower or higher tercile of the normal range were not (RR=1.39, 95% CI 0.98-1.97; RR=0.99, 95% CI 0.76-1.29, respectively) (Wu, Pei et al. 2016). In conclusion, although the link between thyroid function within the reference range and cognitive changes has some inconsistencies, current data seem to support a link between both higher FT_4 and lower TSH levels, with cognitive dysfunction and dementia risk, especially in older age.

Intervention studies

Bipolar and some mood disorders have been demonstrated to improve under levothyroxine (LT_4) treatment also with positive cognitive outcomes (Bauer, Berghofer et al. 2002, Walshaw, Gyulai et al. 2018). But only few *intervention* studies evaluating thyroid function and cognitive outcomes are available in patients with no prior psychiatric dysfunction.

Hypothyroid patients treated with LT_4 were initially evaluated in small anecdotal reports; one reporting a slight positive effect on verbal fluency after 6 months of supplementation (Pollock, Sturrock et al. 2001, Bono, Fancellu et al. 2004). The sole real randomized controlled trial involving clinical hypothyroidism was published by *Walsh et al.* (Walsh, Ward et al. 2006). In this study, 56 subjects (93% female) were randomized to three different LT_4 doses in a crossover design, aiming to achieve three distinct serum TSH intervals for at least 8 weeks. After an expected proportional

response in TSH levels and other biomarkers of TH action, cognitive assessment was made using a battery of tests (Symbol Digit Modalities Test, Trail Making Test Parts A and B, Digit Span Sub-test, and Wechsler Adult Intelligence Scale III) which allowed comparisons over memory, attention and executive function domains. No significant difference was observed between groups in any of these neurocognitive tests. Nevertheless, final TSH levels were all within "normal" or "mild thyrotoxicosis" range, so it is possible to conclude that this study was underpowered to detect any small changes associated to such a slight difference in thyroid function status.

Restoring euthyroidism in *subclinical hypothyroid* individuals also failed to improve cognition function in three placebo-controlled trials. Two of them were small and had important limitations, such as extensive exclusion criteria (that resulted in "treating" a very "healthy" selected population), and a high percentage of patients with "transient elevation of TSH", which could have led to a higher recruitment rate of euthyroid individuals (Jorde, Waterloo et al. 2006, Parle, Roberts et al. 2010). The Thyroid Hormone Replacement for Sub-clinical Hypothyroidism (TRUST trial) was a more robust trial published in 2017 (Stott, Rodondi et al. 2017). In this double-blind randomized placebo-controlled trial, 737 adults with mean age of 74.4 years with persisting sub-clinical hypothyroidism were randomized to placebo or LT_4 aimed to obtain TSH levels within the reference range. After a minimum 12-months follow up and a mean daily dose of 50 μg, the LT_4 group presented no benefit regarding executive cognitive functions, assessed by speed processing letter-digit coding test. On the other hand, two other small studies have reported a positive effect of LT_4 supplementation in subclinical hypothyroid individuals. A randomized-control trial included 37 patients with subclinical hypothyroidism (defined by TSH levels above 6.0 mIU/l on at least two occasions and normal total T_4) for at least six months of treatment and found a marginal improvement in the memory composite score in the active intervention group (Jaeschke, Guyatt et al. 1996). In a more recent brain imaging cohort, six months of non-randomised replacement treatment in 16 subjects with subclinical hypothyroidism lead to significant improvement in spatial working memory tests (memory quotient and accuracy of the n-back task) (Yin, Liao et al. 2013). In line with these publications a reduction in working memory performance was also observed when, in a proof-of-concept study, a short-term subclinical hypothyroidism was induced by partial reducing LT_4 dosage in 16 previously stable supplemented hypothyroid individuals (Gobel, Gottlich et al. 2018). Nevertheless, due to important limitations, there is still no definitive answer about the true effect of LT_4 over memory domains in subclinical hypothyroid individuals.

The influence of induced *thyrotoxicosis* or subclinical thyrotoxicosis status over cognitive function was examined by several authors. Neurocognitive performance was first evaluated by *Botella-Carretero et al.* in a cohort of 18 women with differentiated thyroid cancer patients under chronic suppressive doses of LT_4 who had a worse performance than euthyroid controls in immediate memory and auditory attention tasks (Botella-Carretero, Galan et al. 2003). In a similar case-control study, patients under suppressive doses of LT_4 (n = 31, 28 women) also did worse in executive functions, working memory, psychomotor speed and attention, when compared to matched controls (Jaracz, Kucharska et al. 2012). On the contrary, when evaluating similar thyroid cancer cohorts under suppressive LT_4 treatment (n = 24, all female) *Samuels et al.* found no differences in executive or memory scores, despite some marginal decrease in mental health subscale in the LT_4 group (Samuels, Kolobova et al. 2014). A small experimental design study induced a brief "mild" thyrotoxicosis in previously healthy male individuals through 8 weeks of 250 μg LT_4 per day. This resulted in some significant changes in attention tests but not in executive or memory tasks (Gobel, Heldmann et al. 2015). Induced subclinical thyrotoxicosis effect on working and verbal/visual memory was tested in a double-blinded, randomized, cross-over study of usual LT_4 dose (euthyroid arm) *versus* higher dose (subclinical thyrotoxicosis arm) in 33 hypothyroid subjects (31 women) (Samuels, Schuff et al. 2008). After 12 weeks in each arm, some borderline improvement in mental health subscale (Short form 36) was observed but no differences were found on declarative or working memory scores. It is not known if these distinct results on memory and attention outcomes are related to any gender or morbidity impact on TH and its influence on cognitive processes. The high heterogeneity found between all these studies and the shortage of large cohorts with wide-ranging neurocognitive assessment weakens any definitive conclusion about the effect of exogeneous thyrotoxicosis on cognition.

To summarize, major alterations in cognitive function appear to occur if TH deficiency is present during key periods of brain development. Currently it is unclear if *subclinical* thyroid dysfunction in adults is related to any impairment in cognitive performance during ageing. It is possible that, if deficits in memory and executive function are present, a potential beneficial effect of "normalizing" thyroid function to its age-reference range could be detected in some cohorts. Nevertheless, when exploring connections between neurocognitive performance and thyroid status it is important to note that a single determination of TSH may be inadequate for a clear view about the HPT set-point of each individual and that serum concentration of TH may not mirror their actual levels in the brain.

Table 1.10. Relation between TSH and FT_4 levels, and distinctive neurocognitive features.

Neurocognitive feature		TSH	FT_4	Reference examples
Global cognition		INVERSE (HypoT range) POSITIVE (HyperT range)		Resta, Triggiani et al. 2012; Kumar, Rana et al. 2019;
		NEUTRAL	NEUTRAL	Roberts, Pattison et al. 2016;
Cognition decline during ageing		POSITIVE (HyperT range)		Kalmijn, Meha et al. 2000;
		NEUTRAL	NEUTRAL	Wijsman, de Craen et al. 2013; Volpato, Guralnik et al. 2002;
		NEUTRAL	INVERSE	Yeap, Alfonso et al. 2012;
Executive function				
	Working memory	INVERSE (HypoT range)	NEUTRAL	Gobel, Gottlich et al. 2018;
		NEUTRAL	NEUTRAL	Stott, Rodondi et al. 2017;
		INVERSE		Szlejf, Suemoto et al. 2018;
Memory				
	Semantic	POSITIVE		Beydoun, Beydoun et al. 2012;
	Verbal	NEUTRAL	NEUTRAL	Samuels, Kaimal et al. 2016;
		INVERSE (HypoT range)		Hogevorst, Hupper et al. 2008;
	Visual		POSITIVE	Beydoun, Beydoun et al. 2013;

	INVERSE (HypoT range)		Hogevorst, Hupper et al. 2008;
Attention	POSITIVE (HyperT range) INVERSE (HypoT range)	INVERSE (HyperT range)	Botella-Carretero, Galan et al. 2003; Constant, Adam et al. 2005;
	NEUTRAL	NEUTRAL	Samuels, Kaimal et al. 2016;

HyperT, hyperthyroidism; HypoT, hypothyroidism.

1.8 GH/IGF-1 axis

The GH/IGF-1, or somatotropic, axis in mammals comprises three main levels (Melmed 2017). At the hypothalamic level, neurons with special neurosecretory nerve terminals localized in hypothalamic arcuate, ventromedial and peri- or paraventricular *nuclei* release growth hormone–releasing hormone (GHRH, somatoliberin) and somatostatin into the hypothalamus-hypophyseal portal system. From there, these peptides reach the anterior pituitary gland level and, respectively, stimulate or inhibit somatotrophs's production and secretion of growth-hormone (GH, somatotropin). Finally, at the third level, peripheral target-organs produce tropic factors (mainly in the liver), like the insulin-like growth factor 1 (IGF-1), in response to GH stimulus. Neuroendocrine regulation of GH secretion is controlled by a classic negative feedback mechanism with three retro-negative hormone loops. GHRH and somatostatin autocrine control over hypothalamic neurons and GH autoregulation over pituitary somatotrophs (ultra-short feedback loop); direct GH effect on hypothalamus GHRH and somatostatin release (short-loop feedback); and finally, IGF-1 action (and several other circulating factors also influenced by GH) at pituitary and hypothalamus level (long-loop feedback) (Figure 1.14). A more complex neural, and hormonal, open-loop regulatory system is also involved in pituitary GH secretion with several factors associated to body metabolic and nutritional status described to influence hypothalamic GHRH and somatostatin secretion (Molina 2018). For example, glucose and non-esterified fatty acids decrease GH levels, whereas arginine stimulates GH release. Hormones like ghrelin, cortisol, T_4/T_3, sexual steroids, insulin, glucagon, leptin and catecholamines can also affect somatotrophs function through a direct or indirect (hypothalamic) action (Giustina and Veldhuis 1998, Steyn, Tolle et al. 2016). Stimulus, like acute stress, that result in a surge of cortisol, catecholamine, and possibly ghrelin, may also lead to acute stimulation of GH secretion. Inversely, hypothyroidism and chronic glucocorticoid excess is known to blunt normal spontaneous GH release.

The somatotropic axis relevance is easily recognized given the abundance of GH-secreting cells in the pituitary level (~50% of all anterior pituitary cells), its functional conservation across all mammalian species and the complex interactions with multiple neuro-hormonal inputs (Melmed 2017). Much like other hypothalamus-pituitary axes, human GH secretion is also pulsatile with 3 main distinct patterns: a true circadian rhythm over a 24-hour period, a basal underlying ultradian pulse and an acute secretagogue-induced activity. Diurnal GH pulsatility is highly dependent on age and nutrition status, but in young healthy individuals, its circadian rhythm is characterized by a

greater nocturnal release that is independent of sleep onset (Steyn, Tolle et al. 2016). Most of the time GH levels are undetectable, with an ultradian rhythm of 10-20 secretory pulses per day, higher in women, and a GH secretory activity facilitated when slow-wave sleep coincides with the circadian cycle. An increase pulse frequency is also observed during night-time with a GH peak usually present within an hour after the onset of deep sleep. This pulse frequency is increased in women, but overall daily GH secretion (associated to nocturnal pulses) is much greater in men. Hypothalamic somatostatin appears to play a leading role in regulating pulsatile GH secretion, higher in men than in women, and this difference is considered a key factor for some of this gender dimorphism (Giustina and Veldhuis 1998).

GH is a 191-amino acid polypeptide that circulates in the plasma mostly attached to two main GH-binding proteins (GHBPs) (around 60%), with high or low affinity (Schilbach and Bidlingmaier 2015). By binding to GHBPs, GH is able to prolong its plasma half-life, reduce its binding to cell membrane receptor (GHR) and provide less vulnerability to acute GH plasma fluctuations. At the cellular level, GH actions start by binding to GHR which belongs to the same superfamily of prolactin and several cytokine receptors. It is present in many biological tissues and cell types, including liver, adipose tissue, bone, brain, muscle and immune system cells. GH binding results in GHR dimerization and is followed by a signal transduction mediated largely by the *Janus* kinase 2 (JAK2) pathway (Pilecka, Whatmore et al. 2007). This GH binding leads to phosphorylation of a variety of intracellular substrates, including the GH receptor itself, and activates several intracellular signalling cascades in particular the signal transducers and activators of transcription (STAT) signalling pathway (especially STAT1, STAT3 and STAT5), the Ras/extracellular signal-regulated kinase (ERK) and the phosphoinositide 3-kinase/protein kinase B (PI3K-Akt) (Dehkhoda, Lee et al. 2018). Activated STATs translocate to the nucleus where they act as transcriptional regulators at specific regulatory DNA response elements. Although many STAT members are activated by JAK2 following GH activation, STAT5 is the predominant transcription factor that delivers most of GH actions and GH-induced cell proliferation (Brooks, Wooh et al. 2008). Deactivation of GH signalling is tightly controlled and occurs at various levels of the signalling cascade. This feature may help explain why many tissues respond only to GH pulses rather than to the absolute GH secretion. One example is IGF-1 liver production, which is enhanced in response to GH-signalling STAT5 activity and has greater activity in response to higher GH pulsatility (Woelfle, Chia et al. 2003). GH is recognized as the major endocrine supervisor of postnatal somatic growth and, *via* IGF-1, it also plays an important role in regulating development and function of different organ systems. Among

the several direct actions induced by GH are the promotion of longitudinal bone growth in children, stimulation of muscle protein synthesis, increased lipolysis, antagonism of insulin action (after a first short-term insulin-like effect), sodium retention, maintenance/development of the immune system and regulation of some brain functions (see below – *GH/IGF-1 axis and cognition*) (Molina 2018).

The main mediator of GH anabolic and mitogenic actions is the IGF-1 peptide. IGF-1 is one of the few polypeptides that share structural similarity to pro-insulin (the others being IGF-2 and relaxin) and is able to bind, although with low affinity, to the insulin receptor (hence the denomination of insulin-like growth factor) (Molina 2018). It is a small peptide mostly synthetized by the liver in response to circulating GH, corresponding to 70% of plasma IGF-1. It circulates at higher plasma concentrations than insulin and acts as a true endocrine hormone, even though it may also be produced and secreted by many other tissues, as an auto- or paracrine modulator (D'Ercole, Applewhite et al. 1980). IGF-1 circulates in the blood either free or bound to specific binding proteins (IGF-binding proteins – IGFBPs) (Ohlsson, Mohan et al. 2009). Six IGF-1 binding proteins (IGFBP-1/6) are currently recognised. They generally attach to IGF-1 and regulate its availability to to bind to its receptor in target tissues. Most circulating IGF-1 is carried by IGFBP-3 in a stable complex that contains a binding protein, IGF-1, and an acid-labile subunit. IGFBP-3 synthesis is highly GH-dependent but it also responds to several hormonal influences such as T_4, testosterone and oestrogen (Clemmons 2018). Contrary to other IGFBPs, IGFBP-3 primary function is to increase IGF-1 half-life by stabilizing IGF-1 binding to this ternary complex in plasma. With much less affinity for IGF-1, IGFBP-2 (the second most common IGFBP) has an important role in the regulation of circulating free IGF-1 but also in helping capillary wall cross and IGF-1 deliver into the extravascular space (Bach 2018).

IGF-1 exerts its action by binding the cell surface IGF-1 receptor (IGF1R). The activation of this ubiquitous high affinity IGF1 tyrosine kinase receptor triggers a conformational change and initiates several intracellular signalling cascades. This includes phosphorylation of the insulin receptor substrate-1 and -2 (IRS-1 and -2), which may also be phosphorylated by the insulin receptor, with subsequent activation of PI3K-Akt and the MAPK pathways; and the activation of Shc (Src-homology-2-containing protein) kinase, which is further linked to the RAS-MAP kinase pathway (Werner, Weinstein et al. 2008). Cellular signalling through these cascades help regulate many downstream effectors, like mTOR and FOXO (Taniguchi, Emanuelli et al. 2006). These, in turn, will control important cellular functions like cell growth, apoptosis, oxidative stress, autophagy, stress

resistance, cellular differentiation and lifespan (Barzilai, Huffman et al. 2012). Because of these numerous IGF-1-mediated transduction mechanisms, a large range of different physiological processes are regulated in distinct tissues by the GH-IGF-1 axis (Holly and Perks 2012). In agreement with the above mechanisms, IGF-1 is a well-recognised enhancer of cell proliferation and differentiation, muscle glucose uptake (much like insulin), bone formation, DNA and protein synthesis, neuronal survival and myelin synthesis.

An important issue to consider is that both GH and its main peripheral mediator (IGF-1) do exert synergism on somatic growth but they are also known to induce opposite effects when considering lipid and glucose metabolism. These contradictory metabolic effects make it sometimes difficult to distinguish physiologic responses to GH and IGF-1 and their independent influence over human lifespan and several other complex biological mechanisms like cognition.

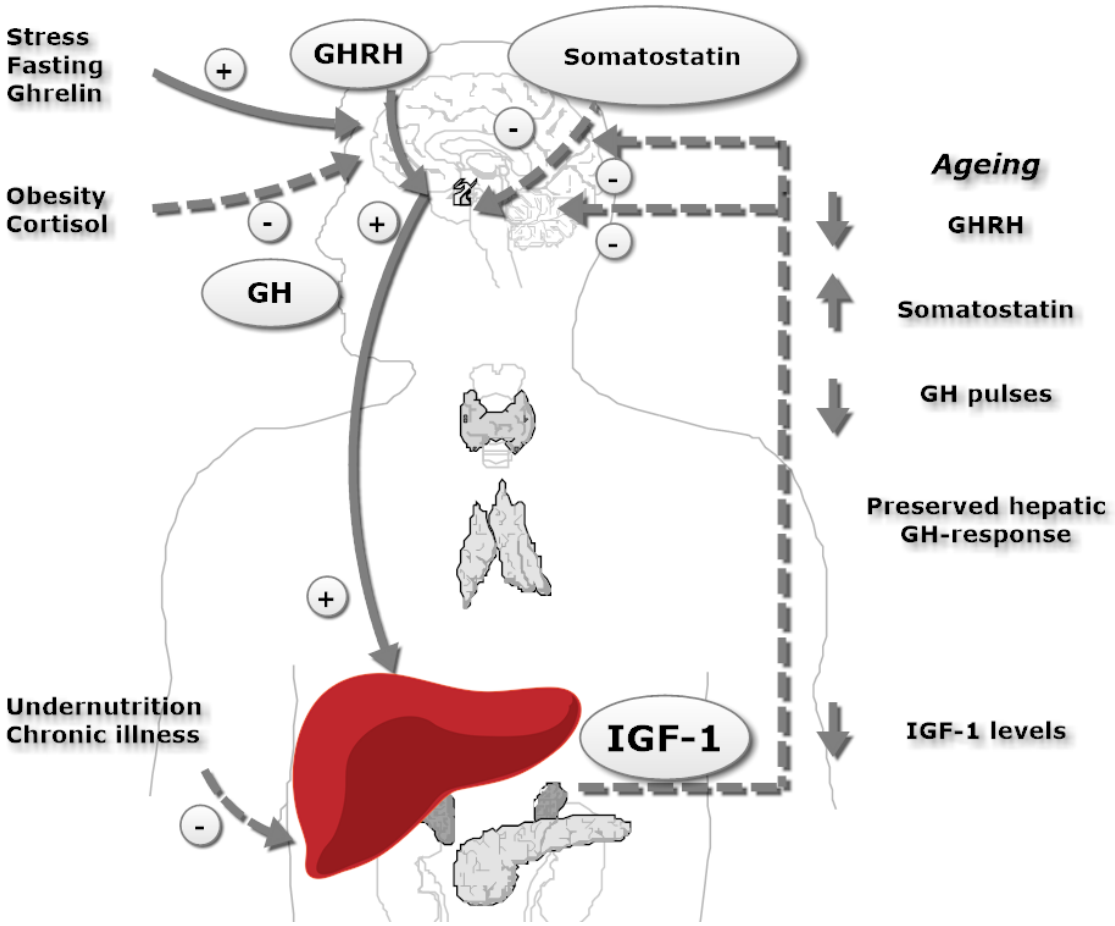

Figure 1.14. Human GH/IGF-1 axis and hormone secretion in ageing individuals.

Defining GH/IGF-1 status

Defining individual GH/IGF-1 status is challenging. GH is a family of polypeptides with over 100 variants in isoform and aggregate combinations. The most frequent (~85%) and classically measured is the 191- amino-acid single-chain 21.5-kDa variant (Melmed 2017). Under normal physiological circumstances, human GH serum half-life is of about 6 to 20 minutes. Serum levels are usually below detection limits in between pulses (usually less than 0.04 ng/ml) and peak concentrations range from 4 up to 40 ng/ml during secretory bursts (Hartman, Faria et al. 1991). Because of this pulsatile and wide nature of secretion, a single random sample for GH measurement is almost always unhelpful to determine individual somatotropic status. Furthermore, despite an overall predictable circadian and ultradian cycle, GH serum levels depend on several physiological inhibitory and stimulatory stimuli, like food ingestion, physical activity and sleep, which will in turn further increase its irregular course. In conclusion, the most accurate way to evaluate pituitary GH secretion is a complete 24-hour period estimation with blood sampling every 5-20 minutes (Giustina and Veldhuis 1998). This will result in an integrated 24-hour average serum GH concentration, that eliminates peak or trough levels that might "corrupt" analysis based on single random sampling of GH. As expected, this methodology is impractical to perform in large population studies.

Serum IGF-1 levels may present a more reliable and stable measurement of GH/IGF-1 status. Under physiologic conditions, free IGF-1 (*i.e.* approximately 1% of circulating IGF-1) and its main blood transporter IGFBP-3, have relatively short half-lives of 10 to 30 minutes, respectively (Clemmons 2012). When bound together along with the acid labile subunit (which represents 80% of total IGF-1 in circulation) half-lives become substantially prolonged, up to 16 hours (Rajaram, Baylink et al. 1997). Total IGF-1 serum concentration is also highly dependent on age, gender and caloric or protein intake. This feature is crucial for evaluating correctly the circulating IGF-1 levels. Normal IGF-1 levels in young healthy adults vary between 91-272 ng/ml in men and 82-261 ng/ml in women (Bidlingmaier, Friedrich et al. 2014).

Immunoassays are the most common technique to measure circulating total GH and IGF-1 levels. However available assays employ distinct methodologies, laboratory protocols and reference ranges, which frequently result into wide differences of GH and IGF-1 determinations and low consistency between different laboratories (Bidlingmaier and Freda 2010, Frystyk, Freda et al. 2010). Recent standardised protocols and reference values have been proposed and published, but are still infrequently employed (Clemmons 2011, Bidlingmaier, Friedrich et al. 2014).

The levels of IGFBP-3 are also frequently measured in order to obtain the IGF-1/IGFBP-3 ratio. This ratio has been suggested to be a surrogate marker of free IGF-1 levels, as the course of their molar ratio, under normal physiological conditions, reflects free IGF-1 levels trend (Juul, Main et al. 1994). Nevertheless, IGFBP-3 is also subject to big variations that are independent of GH/IGF-1 axis activation and respond to gender, adiposity and ethnicity differences (Faupel-Badger, Berrigan et al. 2009).

Somatotropic axis performance may be also explored by provocative tests. An acute GH secretion response may be verified using stimulatory and inhibitory tests, which were primarily created to diagnose clinical conditions of GH deficiency or excess (Melmed 2017). The most frequent stimulatory tests require administration of drugs such as GHRH, glucagon, arginine or insulin. All of them have been used for clinical purposes with various guidelines and cut-points for normal GH response (Hazem, Elamin et al. 2011). The exercise provocative test is a safe non-pharmacologic stimulatory test that explores the physiologic effect of physical activity over GH secretion, probably mediated by cholinergic mechanisms (Casanueva, Villanueva et al. 1984). With an appropriate exercise, basal serum GH levels are expected to increase above the 7 ng/ml threshold and up to 20-30 ng/ml for approximately 100–150 min in young adults, with a weaker response in older individuals (Pyka, Wiswell et al. 1992, Weltman, Weltman et al. 2006). Low levels of diurnal GH secretion are also thought to be a result of daytime food intake. (Cappon, Ipp et al. 1993). Therefore, one of the inhibitory tests used for the diagnosis of GH hypersecretion is based on the Oral Glucose Tolerance Test (OGTT). After an overnight fast, ingestion of 75 to 100 g of glucose will cause a suppression of serum GH to below 0.4 ng/ml within the following two hours (Katznelson, Laws et al. 2014). Probably through somatostatin modulation, after this rapid inhibitory effect of glucose on GH secretion, serum GH levels follows a rather paradoxically rise for 3 to 5 hours after oral glucose administration (Giustina and Veldhuis 1998). Both tests (exercise provocative and oral glucose suppressive) are considered safe and can translate better real-life GH/IGF-1 physiological fluctuation. Despite this “benign” profile their use in population-based studies have been almost non-existent.

GH/IGF-1 axis and ageing

Since the 1920’s, when Evans and Long first reported that administration of bovine anterior pituitary extracts increased growth in rats, it is known that in mammals, pituitary GH and its

intermediary IGF-1 are the main hormones that promote somatic growth during early life and puberty (Evans and Long 1922). Age related changes of GH/IGF-1 axis have been a topic of great interest since the 1980's. Pioneer studies first described a progressive decline in GH serum levels starting early in adulthood among several animal models and paved the idea that GH/IGF-1 axis status could be a marker of the ageing process (Sonntag, Steger et al. 1980, Muller, Cella et al. 1993). Human studies soon replicated some of these findings. Aged individuals secrete less GH during fasting or sleep, and present a significant decline in the number and amplitude of pulsatile GH bursts (Giustina and Veldhuis 1998). These changes result in a continuous reduction of total and pulsatile 24-hour GH production of about 15% for every decade after the second decade of life which culminates latter in circulating GH levels similar to those seen in GH deficiency syndromes (Iranmanesh, Lizarralde et al. 1991, Veldhuis 2013) (Figure 1.15). This fall-out of GH secretion observed during ageing is due to several reasons. Current evidence points out for an extra-pituitary cause like GHRH or ghrelin decreased secretion, increased somatostatin production or even contributions from changes in the HPA axis (Veldhuis, Norman et al. 2012). As discussed above (see *HPA axis and ageing*) during senescence there is a presumptive age-related increase in central CRH or AVP response with rise in overall HPA activity and subsequent hypo-somatotropism, through GHRH suppression by long-term glucocorticoids overexposure (Giustina and Veldhuis 1998, Veldhuis, Keenan et al. 2005). Recognition of HPA axis impact in GH/IGF-1 activity during ageing could help unify some of the many complex endocrine features associated to old age.

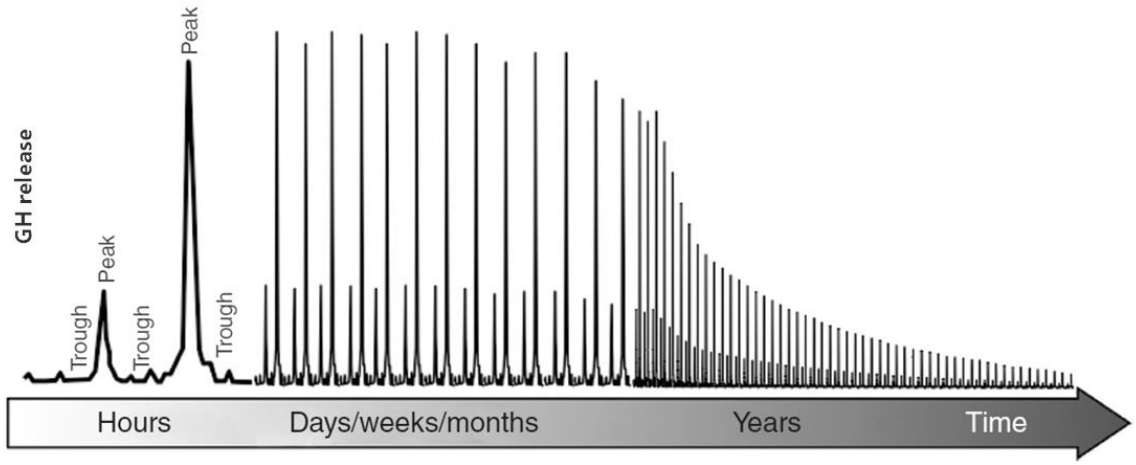

Figure 1.15. GH secretion patterns and their evolution during ageing. Adapted from *Steyn, Tole et al. 2016*.

Parallel to this overall reduction in GH output, IGF-1 levels also decline in a steady, but less pronounced way. In the 7th decade of life, most individuals present a serum IGF-1 concentration 20 to 80% below reference levels for young healthy adults (Corpas, Harman et al. 1993, Maggio, Ble et al. 2006). This age-related relative GH and IGF-1 deficiency is described by many as *somatopause*. Yet, whether this decline represents an adverse or beneficial homeostatic response during healthy ageing is not known. Reduced GH/IGF-1 activity is considered to contribute to some phenotype features found in elderly individuals. Decrease in lean body mass and physical fitness, increased waist-to-hip ratios and reduced quality of life are commonly present in both young persons with GH deficiency and in older healthy individuals (Veldhuis 2013). Clinical trials with GH supplementation in structural GH-deficient patients led to reductions in total-body fat and relevant gains in bone and muscle mass with a proportional elevation in IGF-1 serum levels (Salomon, Cuneo et al. 1989, Rosenfalck, Maghsoudi et al. 2000). Based on this perception, several authors tried to demonstrate this same effect in healthy older individuals by increasing their GH/IGF-1 hormones (Rudman, Feller et al. 1990, Blackman, Sorkin et al. 2002). However, in contrast to young patients with GH deficiency, the potential benefits of GH administration as an "anti-ageing" agent in healthy older people are controversial. Despite some improvement observed in muscle mass, GH treatment was not accompanied by an increase in strength or decrease in fat-mass (specially in women). Moreover, there were troublesome side effects, such as soft tissue oedema, joint pain and new-onset diabetes (Liu, Bravata et al. 2007). Another major concern, due to IGF-1 role on cellular proliferation, is its impact in the incidence and progression of cancer. On this subject, current medical consensus states that *somatopause* is a normal biological status with beneficious effects for the individual and that, until further well-designed studies are conducted, GH-therapy should only be reserved for well documented GH deficiency conditions (Bartke 2019).

Research findings from more than 20-years have also provided evidence that decreased GH/IGF-1 signalling is linked to extended longevity (Taormina, Ferrante et al. 2019). Lack of GH action in animal models, like the GH-deficient *Ames* mice (with a deleterious mutation linked to the pituitary transcription factor PIT-1 gene) or the GH-resistant *Laron* mice (with a disruptive deletion of the GHR) have been associated to a significant increase of lifespan by 20 to 40% (Flurkey, Papaconstantinou et al. 2001). The underlying mechanism has not been fully elucidated but it may share some overlapping features with the caloric restriction model of lifespan extension. As with wild-type mice subjected to caloric restriction (but without malnutrition), GH-deficient and GH-resistant mice are also associated to reduced mTOR signalling (Junnila, List et al. 2013). This

reduction in mTOR signalling is thought to stimulate autophagy, improve cellular response to stress and therefore help explain, at least in part, this increase in lifespan (Wullschleger, Loewith et al. 2006). The mechanism by which GH influences ageing and longevity is probably time-dependent and tissue-specific. In genetic GH-deficient models, GH absence throughout life contributes to extending lifespan and healthy ageing, despite that in some specific target organ GHR knock-out mice models (like those with GHR-KO adipose tissue) a rather opposite effect was also observed (List, Berryman et al. 2013, Bartke, List et al. 2016). Treatment of GH-deficient *Ames* dwarf mice with GH during early life have shortened their longevity significantly (Panici, Harper et al. 2010, Sun, Fang et al. 2017). In agreement with the idea that GH impact on ageing is opposite during different stages of lifetime, some other authors have demonstrated that, in dwarf animal model's, GH signals may have a positive effect on longevity during early development stages, but a negative impact later on in life (Sonntag, Carter et al. 2005). Nevertheless, in humans, several GH-deficient cohorts with dwarfism due to isolated GH deficiency or GHRH receptor mutations failed to observe a rise in life expectancy (Aguiar-Oliveira, Oliveira et al. 2010, Bartke 2019). Likewise, GH-resistance or GH-insensitivity cohorts caused by homozygous mutations in GHR or GH-induced intracellular signalling molecules, also known together as *Laron* syndrome, do not present such extension (Laron, Kauli et al. 2017). Considerable evidence from animal models and human cohort studies with excess of GH/IGF-1 (*i.e.* gigantism or acromegaly) have associated high serum levels of these hormones to greater mortality rate, something that tends to reverse when IGF-1 levels normalize after treatment (Bartke 2003, Ayuk and Sheppard 2008, Holdaway, Bolland et al. 2008).

The separate impact of IGF-1 signalling over longevity has also been explored. By contrast to animal models with GH and GHR deficits, most mice with *null* IGF-1 receptors die at birth. Strains with liver-specific IGF-1 deficiency present better survival rates with an impressive reduction on IGF-1 serum levels despite significant high levels of GH (as a result of long-loop negative feedback action). In spite of IGF-1 decrease in both male and female animals, only a 16% rise in mean lifespan was observed in the female mice (Svensson, Sjogren et al. 2011). Heterozygous animals for IGF-1 receptor deletion have been linked to extended survival, particularly in female mice when decreased IGF-1 tissue bioavailability was more pronounced earlier in life (Holzenberger, Dupont et al. 2003, Ashpole, Logan et al. 2017). In humans, loss-of-function mutations of IGF-1 or IGF-1 receptor genes are associated to severe medical conditions with decreased intra-uterine growth, developmental delay, hypoglycaemia, microcephaly and sensorineural hearing loss (Woods, Camacho-Hubner et al. 1996, Abuzzahab, Schneider et al. 2003). Nevertheless, much of the evidence for a negative

correlation between IGF-1 signalling and human ageing has been provided by epidemiological studies (Suh, Atzmon et al. 2008, van Bunderen, van Nieuwpoort et al. 2010). Low serum IGF-1 levels were associated to an increase longevity in several healthy ageing cohorts (Milman, Atzmon et al. 2014, van der Spoel, Rozing et al. 2015). Interestingly, the offspring of these centenarians were also shown to present an overall reduction in GH secretion and IGF-1 serum concentrations, pointing out for a genetically predisposition to familial longevity, probably based on GH/IGF-1 axis signalling (van der Spoel, Jansen et al. 2016). In agreement, some IGF-1 receptor variants with suppressed downward signalling (such as the IGF1 receptor gene mutations Ala37Thr and Arg407His) and polymorphisms in elements of the GH/IGF-1 signalling pathway, such as AKT1 and FOXO3A genes, have also been found more prevalent in long-lived individuals populations (Pawlikowska, Hu et al. 2009, Tazearslan, Huang et al. 2011, He, Lu et al. 2014, Bae, Gurinovich et al. 2018). Some of these genes associated to GH/IGF-1 function were most likely selected for their essential role on development and promotion of maturation, but their actions later in life were associated to potential detrimental effects on disease risk and survival. This biphasic relationship fits well into the antagonist pleiotropy theory of ageing (see above c*urrent theories about ageing*) where some late-acting deleterious genes are positively selected for their early life benefits. Also in agreement with this hypothesis are the findings that these GH/IGF-1 axis "longevity genes" are also associated to delayed puberty and reduced fecundity (Aguiar-Oliveira and Bartke 2019).

In conclusion, GH/IGF-1 axis link to longevity is highly time-dependent and probably tissue-specific. Within the right time-window its activation may ensure longer and healthier life. Later in the healthy ageing individual, lowered activity GH/IGF-1, like the one seen in *somatopause*, may actually reflect a useful homeostatic response to ensure higher resistance to tumours and stress.

GH/IGF-1 axis and cognition

Currently there is a strong evidence for establishing GH/IGF-1 axis as essential for brain development and function. Initially it was thought that the brain was not subject to any direct action from circulating GH. Despite some local GH production in the hippocampus (where it is up regulated by stress and oestradiol), lateral hypothalamus, dorsal striatum, ventral thalamus and reticular formation, most GH do reach the brain through the blood-brain barriers (Gossard, Dihl et al. 1987, Zhai, Lai et al. 1994, Nyberg and Burman 1996, Donahue, Kosik et al. 2006). In confirmation for the GH importance in mammals' brain, is the evidence of putative GHR in many

areas of the central nervous system, such as the hippocampus, hypothalamus, striatum and parietal cortex; with a changed expression throughout brain development, ranging from higher density of GH presence in earlier stages to progressive decline after adulthood (Lai, Roos et al. 1993, Zhai, Lai et al. 1994, Nyberg and Burman 1996).

Brain morphology and function

GH and IGF-1 are established key regulators of brain structure and function. Current data suggest that these hormones may exert distinctive and somewhat opposite effects during different stages of life.

GH

In GH-deficient mouse models, like the *Snell* mice (linked to pituitary transcription factor PIT-1 mutations) or the "*little mice*" (associated to mutations in the *Ghrh* gene), cerebral size is smaller with reduced neuron number and decreased myelination (Noguchi 1996). There is no consensus on whether this results from an overall reduced body size or rather from a direct effect of GH deficiency during early brain development. Similarly, human patients with childhood GH deficiency syndromes exhibit reduced neuronal connections with decreased white matter integrity of the corticospinal tract, corpus callosum and some degree of microcephaly with significant selective reductions in hippocampus, globus pallidum and left thalamus volumes (van Dam, de Winter et al. 2005, Webb, O'Reilly et al. 2012). The exact mechanism by which GH exerts its function within the brain is not completely clear. Most consensual ideas are that GH will increase IGF-1 production both local and peripherally and that it is this mediator that ultimately acts on brain development and function (Nyberg and Hallberg 2013). Other hypotheses are that GH could directly enhance excitatory synaptic transmission, through GHR or by increasing locally peptide-dependent transmission through GH-degradation into bioactive products which affect major brain neurotransmitter circuitries (Nyberg 2000, Mahmoud and Grover 2006, Devesa, Almenglo et al. 2016). Moreover, GH is also known to be involved in local expression of several cytokines and neurotrophic factors that expand its neuroprotective features, such as erythropoietin, vascular endothelial growth factor and brain-derived neurotrophic factor (Devesa, Almenglo et al. 2016).

IGF-1

While it is uncertain if physiologically relevant concentrations of GH are produced in the central nervous system, significant quantities of IGF-1 are locally synthetized throughout the whole brain (Bondy, Bach et al. 1992, Joseph D'Ercole and Ye 2008). Its paracrine expression reaches a peak perinatally and declines thereafter, persisting during adulthood in some brain regions which are associated to neurogenesis and remodelling, such as the hippocampus, olfactory bulb and cerebellum (Bondy, Werner et al. 1992, Joseph D'Ercole and Ye 2008). Although local production falls soon after birth, circulating IGF-1 is continuously taken up by the brain through all lifespan *via* a constitutive transport system (Fernandez and Torres-Aleman 2012). Similar to its ligand, IGF1R is also widely present in the brain but display a more stable pattern of expression during brain development and maturation. In rodents it is concentrated in the choroid plexus, cerebral cortex, hippocampus, olfactory bulb and cerebellum (Bondy and Lee 1993). Consistent to IGF-1 pleotropic role, IGF1R is expressed in several types of neural cells besides neurons, such as astrocytes, oligodendrocytes and microglia (McMorris, Smith et al. 1986, Ballotti, Nielsen et al. 1987, Bondy and Lee 1993). Recent reports showed that there is a moderate increase in IGF1R transcription in the hippocampus and cerebral cortex during ageing, despite relatively preserved paracrine IGF-1 secretion (Ashpole, Sanders et al. 2015). It is not clear if this represents a compensatory homeostatic mechanism in the setting of less IGF-1 bioavailability or a local response to an undetected injury.

Table 1.11. Key points about GH/IGF-1 axis and brain performance.

- Endocrine and paracrine GH and IGF-1 regulate neurogenesis and brain cell survival
- Constitutive GH and/or IGF-1 "deficiency" may represent a homeostatic response during ageing
- Both IGF-1 low and high circulating levels during adulthood are inversely related to latter cognitive performance
- Some improvement in Executive and Memory tasks is possible after treating GH deficient patients with GH and elderly individuals with GHRH
- Excessive heterogeneity among human studies and technical difficulties in separating GH from IGF-1 effects and systemic from local functions weakens any definite conclusions

Several *in vitro* and animal studies demonstrated that IGF-1 promotes neurogenesis, cell survival, synaptogenesis, neurite growth and myelination, both during brain development and in adulthood (Gubbi, Quipildor et al. 2018). These beneficial effects are crucial for proper brain growth but also in repairing damaged neural tissues during senescence. Increased intracerebroventricular IGF-1,

either by direct infusion or gene therapy, was enough to restore hippocampal neurogenesis and to improve synaptic function in old rats (Lichtenwalner, Forbes et al. 2001, Pardo, Uriarte et al. 2016). Some of these healing features occur within minutes after brain injury and are associated to IGF-1 dependent NMDA receptor activation, as well as to improved neuronal glucose use and acetylcholine release (Nunez, Carro et al. 2003). Some other recovery pathways, such as brain neurogenesis, angiogenesis and synaptogenesis, are more delayed and dependent on prolonged IGF-1 activation (Frater, Lie et al. 2018).

Both surviving homozygous and heterozygous IGF1 or IGF1R-knockout mice present reduced brain size and loss of myelination which affects heavily the hippocampus and striatum areas (Beck, Powell-Braxton et al. 1995). IGF1-knockout mice have also shown overall reduced dendritic length and complexity with a significant decrease in synapse number in the frontoparietal cortex (Cheng, Mervis et al. 2003). In agreement, brain IGF-1 over-expression in transgenic mice models has been linked to bigger brain size and a surge of neuron number and myelin content (Carson, Behringer et al. 1993).

In humans, "pure" IGF-1 deficiency syndromes are rare and associated to smaller brains. Heterozygous mutations in IGF-1 and IGF1R genes are associated to microcephaly and mental impairment but no data on brain structure has been published (Ashpole, Sanders et al. 2015, Juanes, Guercio et al. 2015). In contrast, *Laron* syndrome patients with GHR deficiency (see above *GH/IGF-1 axis and ageing*) present brain structure with larger dentate gyrus and CA1 regions of hippocampus, as well as bigger cortical areas in several frontal and cingulate regions (Nashiro, Guevara-Aguirre et al. 2017). Although the mechanisms for this neuroprotection effect are unknown, it seems that GHR deficiency does not interfere with autocrine and paracrine IGF-1 production and perhaps only increases IGF-1 brain sensitivity, therefore helping preserve a younger phenotype (Guevara-Aguirre, Balasubramanian et al. 2011).

Some structural data have arisen from epidemiological studies. In a large community-based study with close to 2,000 older and middle-aged adults without dementia and evaluated by brain MRI, serum IGF-1 levels were correlated to total brain volume but not to hippocampal volume (Westwood, Beiser et al. 2014). Similarly, a prior study failed to show a significant correlation between pathological wider radial width of the temporal horn (a surrogate imaging marker for medial temporal lobe and hippocampus atrophy) and IGF-1, although this study sample was much smaller (n=75) (Angelini, Bendini et al. 2009). This discrepancy among the link between IGF-1 and

the hippocampus *versus* total brain volume may result from an amplified measurement error (often found when evaluating small structures like the hippocampus), rather than to any variance in the biological IGF-1 effect over these brain regions.

As discussed earlier, IGF-1 has an essential role in the acute brain-injury recovery response. Temporary elevations of IGF-1 have been associated to better outcomes in acute hypoxic-ischaemic brain models, in line with its recognised role in neuronal survival and recovery (Zhu, Fan et al. 2008, Lin, Fan et al. 2009). Nevertheless, such beneficial effect has apparently been lacking in chronic conditions. *Ames* dwarf mice have reduced amyloid plaque deposition and IGF1R-KO neurons present better autophagy and clearance of Aβ plaques (Gontier, George et al. 2015, Puig, Kulas et al. 2016). Local brain IGF-1 resistance with reduced IGF-1 signalling has been also reported in animal models and confirmed in human Alzheimer's disease brains (Talbot, Wang et al. 2012, Trueba-Saiz, Cavada et al. 2013). It remains unknown whether these aspects represent a real pathologic feature or a protective response. Interestingly, this protective role of subnormal GH/IGF-1 signalling was not observed in more advanced Alzheimer disease models, implying that an early time-window is probably needed for its advantageous effect to take place (George, Gontier et al. 2017).

Some contradictory data also emerges from human epidemiological studies. Earlier reports found that circulating IGF-1 was associated to a small increase in risk of progression to Alzheimer's disease (Duron, Funalot et al. 2012, Westwood, Beiser et al. 2014). But in two recent meta-analysis, involving more than 1,000 individuals, there were no conclusive relationships between IGF-1 serum levels and risk of developing Alzheimer's disease (Hu, Yang et al. 2016, Ostrowski, Barszczyk et al. 2016).

In line to this idea of IGF-1 double role in the ageing brain (with a beneficial effect during acute injury response but a negative impact when sustained overtime) there are some results coming from *in vitro* and *in vivo* experiments. In these reports, sustained brain IGF-1 exposure was associated with an increased generation of reactive oxygen species and an inhibition of autophagy and cellular stress responses (Bitto, Lerner et al. 2010, Torres Aleman 2012). Features that are known to reduce brain cellular resilience and increase abnormal accumulation of protein debris, help to explain the apparent paradoxical effect of IGF-1 over age-related neurodegenerative conditions, such as Alzheimer's disease (Gubbi, Quipildor et al. 2018).

The most consensual theory for this apparent low IGF-1 protective effect during brain ageing states that GH/IGF-1 signalling decrease could represent a homeostatic mechanism in which, by reducing the ageing process and limiting the accumulation of debris, brain cells would try to preserve overall neuronal function and shift from cell-proliferation to cell-maintenance. Again, if this age-related GH/IGF-1 axis modification is a compensatory brain adaption or just a surrogate marker of brain damage, is currently unknown.

Neurocognitive performance

Animal studies

Few animal studies have tackled cognition and GH/IGF-1 axis in the context of ageing. While long-lived *Ames* dwarf mice present better memory performances than age-matched controls, mice with astrocyte-specific knockout of *igf1r* gene during early development have impaired working memory throughout adult life, although no data is available from older cohorts (Kinney, Meliska et al. 2001, Logan, Pharaoh et al. 2018).

In traumatic brain injury models, rats treated, for at least 2 weeks, with GH for two months after the trauma event presented better spatial memory recovery (Zhang, Han et al. 2014). This improvement was independent of previous basal serum GH levels.

In healthy rats, prolonged systemic GHRH administration from 9 to 30 months of life also attenuated age-related spatial memory decline, with no changes noted in sensorimotor performance (Thornton, Ingram et al. 2000). Similarly, several intervention studies demonstrated an improvement in cognitive decline of spatial and working memory in aged rats after increasing intracerebroventricular IGF-1 levels (Markowska, Mooney et al. 1998, Pardo, Uriarte et al. 2016, Pardo, Abba et al. 2018).

In short, and despite some contradictory results, evidence from animal models support the idea that an overall reduced GH action can be beneficial to learning and memory performance throughout life; and that “acute” local rise of GH/IGF-1 is rather useful in recovering from brain damage and age-related deterioration.

Human studies

Since the 1980's, major cognitive deficits have been recognized in GH-deficient children (Abbott, Rotnem et al. 1982). Adults with GH-deficiency display relevant memory impairment. Functional brain MRI studies revealed that GH-deficient patients do present subnormal speed processing but no apparent compromise in overall memory performance, probably through recruitment of dorsal prefrontal cortex resources (Deijen, de Boer et al. 1996, Arwert, Veltman et al. 2005). Importantly, treatment of these individuals with GH resulted in some cognitive recovery (Falleti, Maruff et al. 2006).

GH/IGF-1 axis status and cognition has been explored in healthy older individuals by several cross-sectional and longitudinal studies. Most reports recognized a positive association between serum IGF-1 levels and better performance in global cognition scores. A limitation to these findings was the small number of participants included, low discriminative neurocognitive tests used and single time IGF-1 assessment (some of them decades before neuro-cognitive assessment). Inconsistent data was also observed regarding gender, with significant correlations restricted only to males or females depending on the reports. In two long longitudinal studies evaluating midlife IGF-1 levels and cognitive performance 18-years later, the authors found that both men and women with higher concentration of IGF-1 presented overall better global cognitive scores, although less pronounced in women (Okereke, Kang et al. 2006, Okereke, Kang et al. 2007). In a cross-sectional analysis of 1,535 individuals with median age of 74 years, mean MMSE and verbal fluency scores were better in a dose-response manner for men, but not in women (Al-Delaimy, von Muhlen et al. 2009). On the other hand, in another cross-sectional population-based study of 1,320 middle-aged to older participants, only women with intermediate levels of IGF-1 were found to perform better in global cognition, visuo-spatial and attention tests *versus* female participants with lower or higher IGF-1 serum concentrations. These results questioned the notion of gender effect but, more importantly, proposed an inverse U-shaped relationship between GH/IGF-1 axis status and cognition performance during healthy ageing (Wennberg, Hagen et al. 2018). In agreement with this work, a prospective 8-year follow-up study involving 400 middle-age and older men also recognized that individuals with high or low middle-life IGF-1 levels were associated to worse cognitive performances, suggesting that intermediate levels of IGF-1 were linked to less cognitive decline (Tumati, Burger et al. 2016). These results were not corroborated by other studies. In a Welsh longitudinal cohort of 746 men followed for 20-years, the authors failed to find any direct association between middle-age serum IGF-1 and age-related cognitive decline, cognitive

impairment or dementia (Green, Holly et al. 2014). In another study evaluated an exceptional long-lived cohort (over 95-years of age), *Perice et al.* found that, cross-sectionally, cognitive impairment prevalence was much smaller in the low IGF-1 group *versus* higher concentration, but only in women (Perice, Barzilai et al. 2016). On the contrary, more recently *Paulsen et al.* reported that in 1,970 older men, higher IGF-1 was associated to a decreased 5-year risk of incident mild cognitive impairment/dementia (Paulsen, Schubert et al. 2019). There is still no published meta-analysis that includes only healthy older individuals, probably due to substantial heterogeneities found between different published reports. Nevertheless, despite some mixed evidence, a recent systematic review brought support for a positive association between IGF-1 and global cognition in elderly, stressing the relevant issue of right timing and duration of exposure (Frater, Lie et al. 2018).

Human intervention studies

Several small studies evaluated cognitive outcomes in GH deficient patients supplemented with GH. A meta-analysis reviewing data up to 2005 (4 studies, 85 patients) did not find evidence for cognitive improvement in GH deficient individuals after up to 12 months of GH substitution (Arwert, Deijen et al. 2005). Other parallel meta-analysis that included more cross-sectional studies and some recent publications have questioned this conclusion (Falleti, Maruff et al. 2006). In a small double-blind, placebo-controlled study with 12 childhood-onset GH deficient adults (mean age 28 years), GH treatment for 6 months resulted in improved memory functioning, in long-term and working memory (Arwert, Veltman et al. 2006). On the contrary, in an open-label clinical trial, 32 subjects with GH adult-onset deficiency receiving GH therapy for at least 1 year were randomized to either decrease or increase of daily GH dose. After a period of 24 weeks, there were no differences on the low and high dose GH treatment in males with respect to executive and memory tasks (van Bunderen, Deijen et al. 2018). However, increment of GH dose in female patients resulted in a reduction of working memory functioning, implying that, in women with GH deficiency, GH substitution could have a negative dose-effect. This work relaunched the idea that there is a U-shaped relationship between GH/IGF-1 axis and cognition, where high GH dose appears to impair prefrontal cognitive performance, while a low dose could result in reduced vigor.

A hand full of small clinical trials have explored the role of GH treatment in post-trauma brain injury patients. A minor positive effect on overall cognitive performance was observed in most reports with chronic stable conditions and secondary GH-deficiency (High, Briones-Galang et al. 2010,

Moreau, Cortet-Rudelli et al. 2013). In subacute settings, a recent randomized controlled trial involving 40 adult patients submitted to 6 months of treatment with human-recombinant GH (aiming to IGF-1 target in the upper quintile of the range for age and body weight) failed to identify any effect on cognitive executive function, measured by an extensive neuro-cognitive number of tests (Dubiel, Callender et al. 2018). These data corroborate the assumption that, regardless of aetiology, GH replacement in individuals with no clear evidence of GH disruption will not result in a significative cognitive improvement.

Currently only four intervention trials have been published on cognitive outcomes in healthy older adults. Fifty-two healthy individuals with more than 69 years of age were randomized by *Papadakis et al.* to recombinant human GH *versus* placebo (Papadakis, Grady et al. 1996). After 6 months of supplementation, despite some enhancement in physical ability, no improvement was observed in general measures of cognition and working memory. Similarly, *Friedlander et al.* failed to observe differences on memory tasks after 1 year of IGF-1 replacement therapy in healthy, nonobese, postmenopausal women over 60 years of age (Friedlander, Butterfield et al. 2001). On the other hand, in a randomized, placebo-controlled, double-blinded study with 89 healthy older men and women, GHRH treatment for 6 months resulted in a small (6%) but significant improvement on cognitive functions, particularly those involving problem solving, psychomotor processing speed and working memory in both genders (Vitiello, Moe et al. 2006). More recently, *Baker et al.* performed a large double-blind randomized control trial with 152 individuals (aged 55 to 87 years), 66 of which already with mild cognitive impairment. After 20 weeks of treatment with a human GHRH analogue, executive function cognitive scores were improved both in the healthy group and in the better performers within the mild cognitive impaired group (Baker, Barsness et al. 2012). This trend was also present in verbal memory but absent in visual memory tasks. In general, these interventional human studies show no clear cognitive benefit for interventions on this axis with GH or IGF-1 administration and only a modest positive response after more than 5 months of GHRH administration.

To summarize, animal and human studies have clearly established an interaction between GH/IGF-1 and the central nervous system functioning. Nevertheless, it has been difficult to demonstrate a potential positive effect of GH and IGF-1 over cognition during ageing, mostly because of difficulty in detaching effects of GH from IGF-1, systemic role of GH/IGF-1 *versus* local brain synthesis, and

due to the unique and sometimes opposing effects that these same hormones may exert during different stages of the lifespan. Importantly, although GH "treatment" in healthy elderly subjects is sometimes proposed as an attractive mean to obtain a "healthier" ageing, it has not been associated to any clear cognitive improvement and may in itself potentiate harmful outcomes related to chronic activation of GH/IGF-1 signalling.

Table 1.12. Relation between GH/IGF-1 axis and distinctive neurocognitive features.

Neurocognitive feature		GH	IGF-1	
Global cognition		NEUTRAL (GH-deficient)		Arwert, Veltman et al. 2005;
			POSITIVE	Okereke, Kang et al. 2006;
			INVERSE	Tumati, Burger et al. 2016;
Cognition decline during ageing			POSITIVE	Paulsen, Schubert et al. 2019;
			NEUTRAL	Green, Holly et al. 2014;
Executive function				
	Working memory	INVERSE		van Bunderen, Deijen et al. 2018;
		POSITIVE (GH-deficient)		Arwert, Veltman et al. 2006;
			POSITIVE (intermediate levels)	Wennberg, Hagen et al. 2018;
			NEUTRAL	Green, Holly et al. 2014;
Memory				
	Semantic	POSITIVE (GH-deficient)		Falleti, Maruff et al. 2006;
			POSITIVE (intermediate levels)	Wennberg, Hagen et al. 2018;
	Verbal	POSITIVE (GH-deficient)		
			POSITIVE	Okereke, Kang et al. 2006;
	Visual	POSITIVE (GH-deficient)		
Attention		?	?	

2. Aims and research questions

The main objective of this work was to assess various endocrine axes in healthy ageing individuals and their relationship with cognition during senesce. To accomplish this aim, two cognitive distinct elderly populations were evaluated with emphasis on their vitamin D status, hypothalamus-pituitary-adrenal (HPA), hypothalamus-pituitary-thyroid (HPT) and growth hormone/insulin-like growth factor I (GH/IGF-1, somatotropic) axes.

The central hypothesis was that healthy older individuals tend to present some neuro-endocrine influence over cognitive features.

The study was based on a well neuropsychologically characterized cohort of healthy individuals older than 55-years old and previously clustered as "Good" and "Poor" within normal cognitive age-adjusted performance. The research questions of this thesis were addressed in a longitudinal perspective:

1. Are 25(OH)D levels different between distinct cognitive elderly individuals? Do they relate the diverse processes of cognition during ageing?

2. Is there any difference in the HPA axis, in a steady-state situation, between distinct cognitive elderly individuals? How is the cognitive performance associated by their current HPA axis features?

3. Are their cognitive differences related some HPT axis inequity? Is there any distinction in thyroid hormone levels, or in thyroid morphology, between the two groups? How does cognition performance interact with the HPT axis?

4. How steady-state and physiologic modulation of the GH/IGF-1 axis differs between the two distinct cognitive elderly groups? Has it any influence on the cognitive longitudinal performance observed?

3. 25-OH vitamin D levels and cognitive performance: longitudinal assessment in a healthy aging cohort

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Abstract

Background: Declining serum levels of 25-hydroxyvitamin D [25(OH)D, a biomarker of vitamin D status] with aging is a well-recognized phenomenon. However, scarce information is available on the relation between 25(OH)D levels and cognitive performance over time in older individuals. Our purpose was to evaluate, longitudinally, the association of 25(OH)D with cognitive function in a healthy older adults' cohort.

Methods: Sixty-four individuals over 55 years-old with no cognitive impairment, clustered as healthy "Poor" and "Good" cognitive performers, were followed for an average of 18 months. Seasonal-adjusted 25(OH)D serum levels (measured by high-performance liquid chromatography-tandem mass spectrometry) were related, longitudinally, with cognitive (memory and general/executive) composite scores.

Results: Overall seasonal-adjusted median serum 25(OH)D level was of 47 nmol/l [interquartile range (IQR), 38-60 nmol/l]. A negative correlation between baseline 25(OH)D and the general/executive composite score was found in the "Poor" cognitive performers (r=-0.52, p=0.006), an association lost after adjusting 25(OH)D levels for the season. No effect was found in both groups between seasonal-adjusted 25(OH)D levels and the variation of both memory and general/executive composites during follow-up when adjusted for age, gender and education level.

Conclusion: In this healthy older population with no cognitive impairment, lower serum levels of 25(OH)D were not longitudinally associated with poorer cognitive scores.

Introduction

Vitamin D is a steroid prohormone obtained from the diet or produced by the action of ultraviolet light in the skin (Bouillon 2016). Once in circulation, it is rapidly hydroxylated in the liver into 25-OH vitamin D [25(OH)D]. Levels of 25(OH)D are considered surrogate indicators of vitamin D homeostasis (Bouillon 2016). While its best well-known function resides on the regulation of calcium homeostasis, evidence is accumulating on the association of vitamin D deficiency with reduced musculoskeletal health and increased risk for acute and chronic diseases, as well as all-cause mortality (Pludowski, Holick et al. 2013, Schöttker, Jorde et al. 2014). More so, some studies found significant positive associations between blood 25(OH)D and several cognitive performance scores in different gender and age groups (Annweiler, Schott et al. 2010, van der Schaft, Koek et al. 2013, Anastasiou, Yannakoulia et al. 2014, Granic, Aspray et al. 2015). However, available observational data in older populations, evaluating various cognition domains and 25(OH)D levels, provided contradictory results. Several, but not all, cross-sectional and prospective studies involving adults older than 60 years have shown an increased risk of cognitive impairment for those with low levels of 25(OH)D (Annweiler, Montero-Odasso et al. 2013, Goodwill, Campbell et al. 2018). Still, overall, very few studies have addressed this issue longitudinally in a community-dwelling senior population setting and with a standardized 25(OH)D serum measurement method (Perna, Mons et al. 2014, Kuzma, Soni et al. 2016).

Here the objective was to evaluate the association between cognitive longitudinal performance (average follow-up of 18 months) and 25(OH)D serum levels determined by high-performance liquid chromatography-tandem mass spectrometry. The population sample was comprised of healthy individuals aged 55 years and older, with no cognitive impairment, but characterized by distinct "normal" cognitive performance patterns.

Material and Methods

Subjects

The study was conducted between March 2012 and March 2015. Summarily, the recruitment was performed in two-phases. First, a larger sample, representative of the general Portuguese older population in terms of age, gender, and education underwent a full neuropsychological assessment (subjects were randomly selected from the Guimarães and Vizela local area health authority registries), resulting in 1,051 participants after inclusion/exclusion criteria (Costa, Santos et al. 2013, Santos, Costa et al. 2013, Santos, Costa et al. 2014). Then, of these, 120 subjects (matched

for gender and age) were chosen in order to provide cognitive profiles of overall "good" cognitive performance (n = 60) and overall "poor" performance (n = 60) group, based on their, within normal range, neuropsychological testing. Primary exclusion criteria included inability to understand informed consent, participant choice to withdraw from the study, dementia and/ or diagnosed neuropsychiatric and/or neurodegenerative disorder. Adjusted thresholds for cognitive impairment were calculated depending on factors such as age and/or education (Grigoletto, Zappala et al. 1999, Busch and Chapin 2008). Thus, the applied Mini Mental State Examination (MMSE) test score thresholds were the following: MMSE score < 17, if individual with 4 or less years of formal school education and/or 72 or more years of age, and MMSE score < 23 otherwise (follows the MMSE validation study for the Portuguese population) (Guerreiro 1994). All participants were community dwellers. For final analysis purposes, subjects with prior history of renal failure, cerebrovascular disorders, osteomalacia, any other bone disease, or those who were on calcium and/or vitamin D supplements were excluded. Individuals who presented estimated glomerular filtration rate below 50mL/min/1.73m^2 and/or who did not have 25(OH)D serum concentration data available were also excluded. The final sample for consideration to the longitudinal analysis was of 64 participants, all of them attended the complete evaluation sessions and had a "seasonal-adjusted" vitamin D evaluation (Figure 3.1).

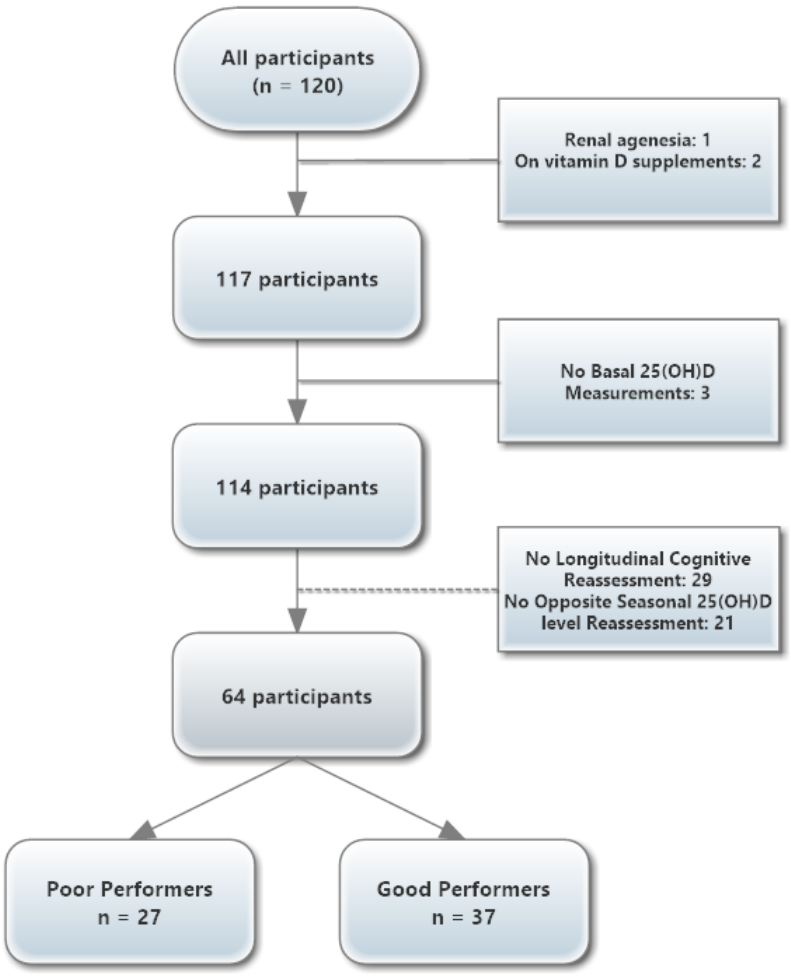

Figure 3.1 Overview of the participant flowchart.

The study was conducted in accordance with the Declaration of Helsinki and approved by national and local ethics review boards. All study goals and nature of the tests were explained to the potential participants and informed signed consent obtained.

Analytical methods

The season of blood collection was dichotomized into Winter-Spring (between 21st December and 20th June) and Summer-Autumn (between 21st June and 20th December), based on the usual solar solstices/equinoxes' dates. All subjects underwent fasting for morning blood collection. Blood samples were collected, centrifuged and stored at -20°C until 25(OH)D levels determination. A seasonal-adjusted mean 25(OH)D levels was obtained for each participant with two blood samples performed on a different season collection (Winter-Spring and Summer-Autumn) over a 1 to 2 years period follow-up to create an individual "season-adjusted" value (n=64). 25(OH)D measurement was obtained by a high-performance liquid chromatography-tandem mass spectrometry (LC-

MS/MS) method (Waters Corporation, Milford, Massachusetts U.S.A.) with a coefficient of variation of 4.9%. As expected (since individuals under supplementation were excluded from the study), 25(OH)D2 levels were negligible (corresponded to less than 1% of the full D2+D3 concentration). Therefore, total 25(OH)D levels reported here correspond to the 25(OH)D3 determination.

Vitamin D deficiency definition based on serum 25(OH)D values is not consensual. For description purposes we categorized into 3 groups based on: 30 nmol/l (12 ng/ml), 50 nmol/l (20 ng/ml) and 75 nmol/l (30 ng/ml) 25(OH)D cut-point levels ("deficiency", "adequacy" and "optimal" thresholds, respectively) (Holick, Binkley et al. 2011).

Vitamin D intake

Vitamin D intake was assessed by a 24h diet recall questionnaire. This estimation was performed using the Nutrilog® software (Nutrilog SAS, France), resorting to the release 23 of the United State Department of Agriculture National Nutrient Database for Standard Reference and adapted to the Portuguese foods using the Portuguese Food Composition Database (INRJ 2007). Food vitamin D fortification is not commonly practiced in Portugal (Barroso 2014).

Cognitive assessment

A team of trained psychologists performed the cognitive/neuropsychological assessments. A test battery was used for socio-demographic characterization and to evaluate multiple neuropsychological dimensions, including cognition profiles [general cognitive status and executive (EXEC) and memory (MEM) functions], as previously reported (Costa, Santos et al. 2013). Briefly, these included: Graffar socio-demographic scale, digit-span forward and backward test, Stroop color and word test, controlled oral word association test (COWAT), selective reminding test (SRT), digit symbol substitution test (DSST) and MMSE (scores adjusted for cognitive impairment and Portuguese population) (Guerreiro 1994). A Principal Component Analysis was performed in order to allocate the multiple test variables into composite components/dimensions, as previously reported (Santos, Costa et al. 2013). Summarily, this resulted in the identification of significant dimensions: memory (MEM) (SRT test variables: consistent long-term retrieval, long-term storage and delayed recall) and general/executive function (EXEC) (COWAT letters F-A-S admissible parameter; Stroop parameters: words, colors and words/colors, digits parameters: forward and backward; MMSE). A z-score for the cognitive composite was calculated and used to select normal extreme values and select "Poor" and "Good" cognitive performers. In our population, the

suitability of using this battery of cognitive tests to measure two latent constructs, memory and executive functioning, was previously demonstrated by a longitudinal invariance analysis across the follow-up (Moreira, Santos et al. 2018).

Statistical analysis

All data are presented as the mean (median), standard deviation (SD) (inter-quartile-range, IQR) for normally (non-normally) distributed data. All continuous variables were checked for normality using the Shapiro-Wilk normality test. Unpaired *t*-test and Mann-Whitney U-test were used to compare continuous variables between the two groups, as appropriate. Wilcoxon matched-pairs signed rank test was performed to compare paired variables that failed normality tests. Fisher's exact test was used for categorical variables. The confidence interval of a proportion was obtained by the modified Wald method. Univariate analysis with Spearman's rank correlation was performed to assess linear relationship between 25(OH)D levels and the different cognitive domain scores (MEM and EXEC) and its temporal trends, stratified by cognitive group. A multiple linear regression analysis was conducted to explore the association between seasonal-adjusted 25(OH)D levels and age, gender and vitamin D intake estimation. Similarly, a multiple linear regression analysis was done to evaluate the prediction of Memory/Executive function scores over time by seasonal-adjusted 25(OH)D levels, adjusted to age, gender, baseline cognitive group and education level. Effect size estimates were calculated with Cohen's d and η^2 for continuous variables with parametric and non-parametric comparisons, respectively; *r* for Wilcoxon matched paired test; ϕ coefficient for Fisher's exact test; and R^2 and adjusted R^2 for regression analysis.

All analyses were tested at the 0.05 level of significance and performed using IBM SPSS Statistics, v.21 (IBM, New York USA) and GraphPad Prism, v.6.00 (GraphPad Software, La Jolla California USA). Effect size estimates were evaluated by Cohen's published benchmark classes (small, medium and large) with the following proposed cut-points: 0.2, 0.5 and 0.8 for Cohen's *d*; 0.01, 0.06, 0.14 for η^2; 0.1; 0.3 and 0.5 for *r* and ϕ coefficient; and 0.02, 0.13, 0.26 for R^2 (Cohen 1988).

Results

After exclusions criteria, the final sample included 64 individuals with a mean age of 65 years (SD 8 years], a women ratio of 0.47 (n=30) and a median follow-up of 18 months (min-max: 16-22 months). Median 25(OH)D levels were higher during Summer-Fall when compared to Winter-Spring

season (55 nmol/l *vs.* 43 nmol/l, p=0.01, η^2=0.06). The overall median seasonal-adjusted serum 25(OH)D level was 47 nmol/l [interquartile range (IQR) 38-60 nmol/l] (range 13-114 nmol/l). Total study population characteristics are presented in Table 3.1.

Table 3.1. Study population characteristics by cognitive performance group, n = 64.

	Total (n = 64)	**"Poor" (n = 27)**	**"Good" (n = 37)**	***p***	**Effect size**
Socio-demographic features					
Sex					
Female, n (%)	30 (47)	14 (52)	16 (43)	0.61	0.09
Age, mean (SD), years	65 (8)	67 (7)	64 (9)	0.09	0.04
Education level above 4-yr, n (%)	17 (27)	2 (8)	15 (41)	0.004	0.37
Vitamin D parameters					
Seasonal-adjusted median serum 25(OH)D concentration, nmol/l (IQR)	47 (38 – 60)	47 (40 – 54)	47 (36 – 61)	0.82	0.001
Vitamin D status [serum 25(OH)D levels]					
Below 30 nmol/l ("deficient"), n (%)	10 (16)	3 (11)	7 (19)	0.50	0.11
Below 50 nmol/l ("inadequate"), n (%)	38 (59)	16 (59)	22 (60)	>0.99	0.002
Between 50 and 74 nmol/l ("adequate"), n (%)	19 (30)	9 (33)	10 (27)	0.60	0.07
75 nmol/l or above ("optimal"), n (%)	7 (11)	2 (7)	5 (14)	0.69	0.01
Vitamin D intake estimation, µg/day, median (IQR)*	0.9 (0.2 – 3.1)	0.5 (0.1 – 2.3)	1.5 (0.2 – 5.6)	0.06	0.06
Cognitive scores and trends, Median (IQR)					
Baseline MEM score	0.23 (-0.97 – 0.74)	-0.98 (-1.27 – -0.73)	0.62 (0.35 – 0.98)	<0.001	0.72
Baseline EXEC score	0.09 (-0.84 – 0.81)	-0.89 (-1.05 – -0.72)	0.70 (0.41 – 1.27)	<0.001	0.72
Last MEM score	-0.65 (-1.19 – 0.49)	-1.16 (-1.49 – -0.91)	0.09 (-0.65 – 1.04)	<0.001	0.35

Last EXEC score	-0.17 (-0.94 – 0.89)	-0.96 (-1.26 – -0.55)	0.793 (0.45 – 1.31)	<0.001	0.71
MEM score Trend	-0.30 (-0.71 – 0.13)	-0.19 (-0.45 – 0.19)	-0.44 (-1.23 – 0.11)	0.03	0.04
EXEC score Trend	-0.004 (-0.11 – 0.14)	-0.003 (-0.12 – 0.16)	-0.009 (-0.10 – 0.14)	0.69	0.003

IQR, interquartile range; MEM, memory dimension composite; EXEC, executive function dimension composite.

* n = 63, 27 *vs.* 36.

Overall vitamin D deficiency (below 30 nmol/l) was found in 10 subjects (16%, 95% CI 9-27%), with only 7 participants (11%, 95% CI 5-21%) surpassing the threshold of optimal 25(OH)D serum concentration (≥75 nmol/L). Thirty-eight participants (59%, 95% CI 47-71%) had 25(OH)D levels below 50 nmol/l. These proportions were not different between "Good" and "Poor" groups (Table 1). The estimated median vitamin D intake was 0.9 µg (36 IU) per day [IQR 0.2-3.1 µg (8-124 IU) per day], and not different among cognitive groups (1.5 *vs.* 0.5 µg/day, p=0.06), with a moderate/low practical effect size (η^2=0.06).

On univariate analysis, "Good" performers had higher education (p=0.004, ϕ=0.37) when compared to "Poor" performers. Cognitive domain scores at baseline and final evaluation were different between the two groups (p <0.001), with a high practical effect size (η^2=0.72), for both domains. MEM score tends to decrease over time in both groups but reached statistical significance only in the "Good" group (p < 0.001), with a very high practical effect size (r=0.56). EXEC scores were stable ("Poor": p=0.83, r=0.04; "Good": p=0.53, r=0.10) and not different between "Poor" and "Good" groups (p=0.69, η^2=0.003), with low practical effect size. There was no association between baseline 25(OH)D levels and MEM and EXEC scores (at baseline, last and longitudinal variation) in both groups, except for the first EXEC score obtained in the "Poor" performance group, with a high negative correlation score (r=-0.52, p=0.006) (Table 2). Seasonal-adjusted 25(OH)D levels were not correlated to any MEM and EXEC scores or to its longitudinal variation in both groups (Table 3.2).

Table 3.2. Spearman's rank correlation (r_s) between 25(OH)D levels and MEM and EXEC scores and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 27)		**"Good" (n = 37)**	
	Baseline 25(OH)D	Seasonal-adjusted 25(OH)D	Baseline 25(OH)D	Seasonal-adjusted 25(OH)D
Baseline MEM score	-0.25	-0.06	0.31	0.25
Baseline EXEC score	-0.52[a]	-0.05	0.22	0.16
Last MEM score	-0.21	-0.05	0.20	0.16
Last EXEC score	-0.26	-0.01	0.11	0.08
MEM score trend	0.15	0.12	0.10	0.08
EXEC score trend	0.24	0.27	-0.13	-0.03

[a] $p = 0.006$.

To explore the association between the observed variations in MEM and EXEC scores over time with seasonal-adjusted 25(OH)D levels adjusted for baseline age, gender, cognitive performance and education level, a multiple linear regression analysis was performed (Table 3). In this fully adjusted model, 25(OH)D seasonal-adjusted serum concentration failed to predict cognitive performance over time (MEM score trend, p=0.96, with moderate effect size: R^2=0.19, adjusted R^2=0.12; EXEC score trend, p=0.47, with low effect size: R^2=0.07, adjusted R^2=-0.005). Being a "Poor" performance individual was linked to a less detrimental decay in MEM score over time (β=0.68, 95% CI 0.29-1.08, p=0.001). No other variable evaluated showed a relevant effect on MEM or EXEC score trends (Table 3.3).

Table 3.3. Association between observed Memory/Executive Function scores trend with Age, Gender, Cognitive Group, Education Level and "seasonal-adjusted " 25(OH)D levels predicted by multiple linear regression analysis, (n = 64), (MEM score trend, $p = 0.96$, $R^2 = 0.19$, adjusted $R^2 = 0.12$; EXEC score trend, $p = 0.47$, $R^2 = 0.07$, adjusted $R^2 = -0.005$).

	MEM score trend		**EXEC score trend**	
	β (95% CI)	*p*	β (95% CI)	*p*
Age (years)	-0.01 (-1.95 to 1.82)	0.29	-0.002 (-0.01 to 0.006)	0.60
Gender (Exposure: Male)	-0.04 (-0.43 to 0.35)	0.84	-0.07 (-0.12 to 0.05)	0.25
Cognitive performance (Exposure: "Poor")	0.68 (0.29 to 1.08)	0.001	-0.01 (-0.14 to 0.11)	0.84
Education Level (years)	0.05 (-0.004 to 0.11)	0.07	0.01 (-0.005 to 0.03)	0.15
Seasonal-adjusted 25(OH)D levels (nmol/l)	0.001 (-0.01 to 0.01)	0.96	-0.001 (-0.004 to 0.002)	0.47

Discussion

In this longitudinal study with a healthy non-demented older cohort we found no association between standardized season-adjusted 25(OH)D levels and the variation of the "memory" and "executive" cognitive domains over a median follow-up of 18 months.

An important seasonal effect over cognitive performance is currently recognized, with annual variations in brain function reaching a peak in late summer and early fall and declining in late winter and early spring (Meyer, Muto et al. 2016, Lim, Gaiteri et al. 2018). This observation may help explain some intraindividual cognitive changes that develop at specific times of the year and the importance of seasonal adjusting when exploring cognitive associations with other variables that share circannual cycles. We observed a negative correlation between baseline 25(OH)D values and baseline EXEC score in the "Poor" performers group, but this effect was lost after adjusting 25(OH)D for season. Our observations are in accordance with the few studies that evaluated cognitive performance and behavior in populations exclusively with vitamin D insufficiency like ours. Some authors using a very low 25(OH)D cut-point level (25 nmol/l), have shown a small or no clear positive association with cognitive performance (Aung, Burnett et al. 2006, Annweiler, Schott et al. 2010). Others, using a somewhat higher 25(OH)D threshold levels, have reported better cognitive performance associated with these "healthier" vitamin D levels (Llewellyn, Lang et al. 2011, Annweiler and Beauchet 2014). Recently, a similar study with non-demented German older adults also found that the lowest 25(OH)D quintiles were associated to higher cognitive decline

(based on a Cognitive Telephone screening instrument score – COGTEL) over an average 5-year follow-up time (Perna, Mons et al. 2014). Interestingly, one study reported that this "positive" effect of 25(OH)D concentrations and cognition tests was only present in the sub-group with low number of years of formal education (Assmann, Touvier et al. 2015). In the current study, despite the overall low level of standard school education years, no such relationship was observed. Further studies are required to clarify whether there is a minimal 25(OH)D threshold that provides neuroprotection in still cognitive healthy populations.

Our multivariate analysis reveals no effect of 25(OH)D on the temporal trends observed in the MEM and EXEC composites in both groups. With this regard, it is important to consider that in contrast to most studies reporting association with single neuropsychological tests, here multiple cognitive components were characterized and grouped into a comprehensive neuropsychological domain composite. When considered alone, the various studies performed to date were seldom concordant. Two recent meta-analyses, including 14 longitudinal studies, indicate that lower 25(OH)D levels are indeed associated with some executive dysfunctions (especially on mental shifting, information updating and processing speed) and cognitive decline. However, they do not provide clear evidence for the relation of 25(OH)D levels with episodic memory or whether there is a 25(OH)D threshold/optimal therapeutic window for supplementation to prevent cognitive decline later in life (Annweiler, Montero-Odasso et al. 2013, Goodwill and Szoeke 2017). These same inconsistencies have been reported more recently in larger and longer prospective cohorts. In a large study spanning over 20-years, 25(OH)D levels measured in midlife were not associated to more rapid cognitive decline over the follow-up period (Schneider, Zhao et al. 2018). However, another large US-based cohort submitted to extensive neuro-cognitive tests covering several domains, found that after a mean 5-year follow-up, higher baseline serum 25(OH)D concentrations were linked to a slower rate of decline in a test of verbal fluency (Beydoun, Hossain et al. 2018). Moreover, a very recent study failed to observe any relationship between baseline 25(OH)D levels and cognition or cognitive decline over 2 years of follow-up by evaluating 1499 Puerto Rican participants living in the Boston Area (aged 45-75 years old at baseline) and using a principal-components analysis to quantify the association between 25(OH)D and the longitudinal performance of two major cognitive features: executive function, and memory (Palacios, Scott et al. 2019).

Unfortunately, current published intervention trials addressing vitamin D and cognition have not provided a conclusive answer (Dhesi, Jackson et al. 2004, Przybelski, Agrawal et al. 2008, Dean,

Bellgrove et al. 2011, Stein, Scherer et al. 2011, Rossom, Espeland et al. 2012, Pettersen 2017, Rutjes, Denton et al. 2018, SanMartin, Henriquez et al. 2018, Castle, Fiedler et al. 2019). A recent small intervention trial showed general cognitive status improvement in 16 mild cognitive impaired patients after 18 months of vitamin D supplementation (but not in healthy controls or those already diagnosed with dementia) (SanMartin, Henriquez et al. 2018). Improved visual memory was observed in healthy Canadian adults with "insufficient" baseline 25(OH)D levels (below 75 nmol/l) that were supplemented with high doses of vitamin D (4,000 IU per day) for 18 weeks, without replication in other cognitive domains (Pettersen 2017). More recently, in a randomized controlled trial with 69 overweight/obese postmenopausal women with 25(OH)D levels less than 75 nmol/l and divided in three different intervention doses of vitamin D (600, 2,000 or 4,000 IU per day), only the intermediate group performed better in learning and memory tests after one year supplementation (Castle, Fiedler et al. 2019). These results reflect the complexity behind any potential vitamin D effect on cognition and the need for better designed studies, probably including distinct vitamin D insufficiency status populations, supplement doses, exposures periods, age groups and baseline cognitive performances.

Another important observation of our study pertains with the 25(OH)D status of the individuals in the study population. Levels below 30 nmol/l were found in 16% and below 50 nmol/l in almost 60%. These results provide a less dramatic indication of vitamin D deficiency/inadequacy compared to recently published data on the Portuguese population (levels below 30/50 nmol/l were detected in 40/69% of a nationwide cluster sample of 1500 Portuguese subjects over 65 years old or 38/86% of a subsample of another national cross-sectional study that included Portuguese adults registered in mainland primary health care centers) (Raposo, Martins et al. 2017, Santos, Amaral et al. 2017). Much of the differences reported in 25(OH)D levels are probably based on the season-geography confounder and on methodological procedures used (Cashman, Dowling et al. 2016). In the present study, the LC-MS/MS was used, which provides a more accurate measure of vitamin D status when comparing to some immunological methods used in routine determinations, and this technicality is likely to justify the main differences found between this and other studies coming from similar Portuguese populations (Wallace, Gibson et al. 2010).

Self-reported daily intake of vitamin D data deserves consideration. Despite the limitation of self-reported data, the estimated median vitamin D intake was only of 0.9 μg/day (36 IU/day) (IQR 0.2-3.1 μg/day, 8-124 IU/day), with all subjects evaluated failing to achieve the recommended vitamin D intake (15 μg/day for adults with less than 70 years and 20 μg/day for those older than

70 years) (Aloia 2011). Main dietary sources of vitamin D are meat, fish, milk and cheese. Overall, in our cohort, meat consumption (meat/fish or eggs was present in 54% of the meals with no individuals reporting being vegetarian - data not shown) was relatively small (mean of 145 mg per meal – data not shown). The current Portuguese Food Composition Database states that eggs and meat have generally low levels of vitamin D (between 0.7 to 1.7 μg per 100mg) (INRJ 2007). Vitamin D supplementation in food is not mandatory in Portugal and most "supplemented" dairy products present levels of vitamin D between 0.3 and 1.5 μg per 100ml or 100g (Parreira, Serra et al. 2015). These two facts may imply that, in our sample, even an "usual" daily consumption of 200g of meat/eggs or "supplemented" dairy products, would represent no more than 20% of the recommended intake of vitamin D. Current habitual intakes of vitamin D in most other countries are also low (typically around 1–4 μg/day), meaning that the proposed reference intake value has to be covered mostly by additional vitamin D supplements and/or endogenous synthesis (Brown, Ignatius et al. 2013). This observation suggests that circulating vitamin D levels in these individuals is most likely provided by exposure to sun. It is important to recall that the present study focuses on a population of active healthy individuals living in the community and that despite the tendency observed towards higher vitamin D intake in the "Good" performing group, no such reflection was present on the their circulating 25(OH)D levels.

The study main strengths are grounded on the exhaustive cognitive evaluation performed longitudinally with the construction of coherent executive and memory cognitive domain composites and a two-point season-opposite 25(OH)D levels determination. However, some important limitations of the study should be considered. First, there was a relatively small number of participants available for final exploration with a complete longitudinal cognitive assessment, which decreased the sensitivity to detect any small effect and prevented further analysis comparing distinct groups based on more extreme 25(OH)D distribution. Second, there was a relatively short time between the two cognitive evaluations (min-max: 16-22 months), which may have also impaired the ability to detect subtle intra or inter-individual cognitive time-dependent differences, underestimating potential associations with 25(OH)D levels. Third, despite the two seasonal opposite 25(OH)D determinations separated by at least 1.5-years, no "real" prolonged longitudinal evaluation of 25(OH)D levels was made. This means that individual vitamin D status appraisal may have resulted in a rather 2-year "cross-sectional" estimation and prevented therefore any assessment of its expanded longitudinal effect on cognition. Also relevant is the fact that the study population was almost homogenously vitamin D insufficient. This feature, frequently found in other

studies with older adults, makes it harder to discover any associations given the lack of 25(OH)D levels inter-individual variability found in these cohorts (Annweiler, Souberbielle et al. 2011).

In summary, in this Portuguese elder healthy population, serum 25(OH)D levels were not associated to cognitive domain composite scores for an average 18 months follow-up, in both stronger and poorer cognitive performers. Despite the lack of agreement about what should be the optimal circulating 25(OH)D levels, the conclusions of the present study are limited to the neurocognitive outcomes obtained in older individuals with relatively stable low 25(OH)D levels and during a short longitudinal period. Further studies should address the role of vitamin D during several brain development time frames and its role on cognitive aging.

4. Longitudinal analysis of HPA axis influence on the cognitive performance of a healthy ageing cohort

Abstract

Background: Higher evening cortisol levels and low day-night amplitude are a well-recognized phenomenon's during human ageing. However, conflicting information is available on the relation between these features and cognitive performance over time in older individuals. Our purpose was to evaluate, longitudinally, the association of several steady-state HPA axis metrics with cognitive function in a healthy older adults' cohort.

Methods: Seventy-six individuals over 55 years-old with no cognitive impairment, clustered as healthy "Poor" and "Good" cognitive performers, were followed for 16 to 22 months. HPA axis measurements (morning plasma cortisol, morning plasma cortisol/ACTH ratio and morning urinary spot cortisol/creatinine ratio) were related, longitudinally, with cognitive (memory and executive) composite scores.

Results: A moderately strong negative correlation between morning urinary cortisol/creatinine ratio and executive composite scores was found in the "good" cognitive performers all over the follow-up period (r_s= -0.37, p=0.01, for baseline; r_s= -0.44, p=0.03, between baseline HPA axis status and last EXEC score; and r_s= -0.50, p<0.001, at last parallel evaluation). This association was lost after adjusting to age, gender and education level. No effect was found in both groups between HPA axis hormones and memory composite scores at baseline or on the longitudinal evaluation.

Conclusion: In this healthy older population with no cognitive impairment, despite some univariate evidence of nocturne cortisol deleterious effect on daytime executive tasks, early-morning and nighttime levels of cortisol were not longitudinally associated with cognitive performance.

Introduction

In normal ageing, there is a loss of hypothalamus-pituitary-adrenal (HPA) axis flexibility towards an overall increased production of adrenal cortex hormones (Heaney, Phillips et al. 2012). In healthy elder individuals, the HPA axis adaptability is weakened, turning adrenal stress response less sensitive to the negative feedback which results in higher evening cortisol levels and decreased diurnal-nocturnal amplitude (Kern, Dodt et al. 1996, Liyanarachchi, Ross et al. 2017). The potential influence that this feature has in the age-related cognitive decline is well understood.

Several large observational studies with non-demented older individuals related higher morning cortisol levels with decreased cognitive abilities (Comijs, Gerritsen et al. 2010, Geerlings, Sigurdsson et al. 2015). Other studies showed that lower evening cortisol levels were positively correlated to a better cognitive performance, particularly in executive domains (Stawski, Almeida et al. 2011). However, conflicting data has been published with respect to both observations (Almela, van der Meij et al. 2012, Singh-Manoux, Dugravot et al. 2014). The precise association between cognitive status and cortisol secretion during ageing may be undermined by other factors that impact the HPA axis, such as mood and sleep disorders, but also by several methodological issues (Potvin, Forget et al. 2013, Veldhuis, Sharma et al. 2013).

These somewhat contradictory statements and the lack of representative studies assessing the full HPA axis/cognition relationship within healthy ageing populations prompted us to conduct the current study. Here the objective was to evaluate prospectively the association between cognitive performance and several steady-state basis HPA axis hormones in an older Portuguese community-dwelling population.

Methods

Subjects

The study population is the same described in chapter 3.

For analysis purposes, subjects with prior history of renal failure, cerebrovascular disorders, dementia, or those who were on corticosteroids were further excluded. Individuals who presented estimated glomerular filtration rate (eGFR) below 50mL/min/1.73m2 and/or that did not have both plasma ACTH, cortisol or urinary cortisol concentration available at baseline or last follow-up evaluation (min-max: 16-22 months) were also excluded. The final sample for consideration for the full analysis was of 76 participants (Figure 4.1).

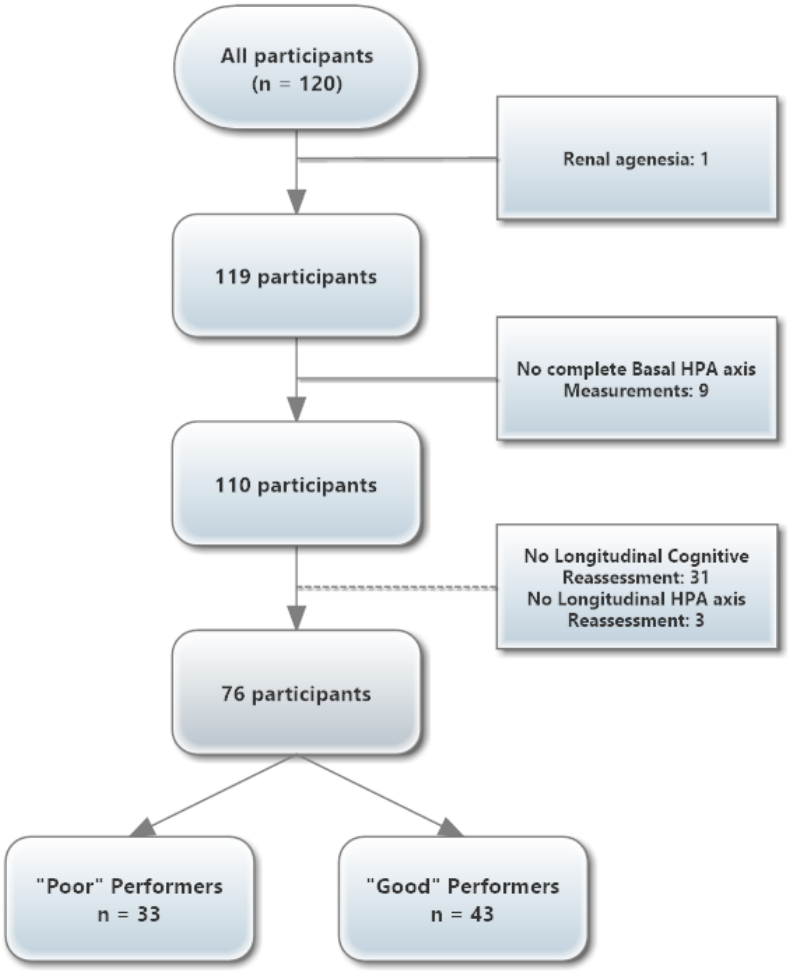

Figure 4.1. Overview of the participant flowchart. HPA, hypothalamus-pituitary-adrenal.

Analytical methods

All subjects underwent fasting for morning blood collection. Blood samples were collected, centrifuged and stored at -20°C until hormone levels determination. First morning urinary samples were collected and stored accordantly. Plasma concentrations of ACTH were measured by Immulite® 2000 chemiluminescent immunometric assay. For analysis purposes ACTH levels below the limit of detection (5 pg/ml) were assumed to be 5 pg/ml. Plasma and urinary cortisol levels were determinate by ADVIA Centaur® chemiluminescent immunometric assay. In order to accurately evaluate morning HPA circadian activation, instead of a more acute stress response, the morning plasma cortisol and cortisol/ACTH ratio (μg/dl *per* pg/ml) was used as a marker of the steady-state morning HPA status (Yang, Megill et al. 2013). Also to minimize stress and night-

time sampling, the morning urinary spot cortisol/creatinine ratio (mg/g) was obtained as a marker of nocturnal HPA production (Corcuff, Tabarin et al. 1998).

Cognitive assessment

As specified in chapter 3.

Statistical analysis

All data are presented as the mean (median), SD (inter-quartile-range, IQR) for normally (non-normally) distributed data. All continuous variables were checked for normality using the Shapiro-Wilk normality test. Unpaired *t*-test and Mann-Whitney U-test were used to compare continuous variables between the two groups, as appropriate. Wilcoxon matched pairs signed rank test was performed to compare paired variables that failed normality tests. Fisher's exact test was used for categorical variables. Univariate analysis was performed to assess linear relationship between different HPA axis status measurements and different cognitive domain scores (MEM and EXEC) using Pearson correlation coefficient or Spearman's rank correlation, as appropriate. A multiple linear regression analysis was conducted to explore the prediction of Memory/Executive function scores and trends by any HPA axis metrics found relevant in univariate assessment with adjustment to age, gender, baseline cognitive group and education level. Effect size estimates were calculated with Cohen's d and η^2 for continuous variables with parametric and non-parametric comparisons, respectively; r for Wilcoxon matched paired test; ϕ coefficient for Fisher's exact test; and R^2 and adjusted R^2 for regression analysis.

All analyses were tested at the 0.05 level of significance and performed using IBM SPSS Statistics, v.21 (IBM, New York USA) and GraphPad Prism, v.6.00 (GraphPad Software, La Jolla California USA). Effect size estimates were evaluated by Cohen's published benchmark classes (small, medium and large) with the following proposed cut-points: 0.2, 0.5 and 0.8 for Cohen's d; 0.01, 0.06, 0.14 for η^2; 0.1; 0.3 and 0.5 for r and ϕ coefficient; and 0.02, 0.13, 0.26 for R^2 (Cohen 1988).

Results

The final sample included 76 individuals with a mean age of 65.1-yr (SD 8.1-yr) and women ratio of 0.47 (n=36). Total study population characteristics are presented in Table 4.1.

Table 4.1. Study population characteristics by cognitive performance group.

Variable	Total (n = 76)	"Poor" (n = 33)	"Good" (n = 43)	*p*	Effect-size
Socio-demographic features					
Sex					
Female, n (%)	36 (47.4)	16 (48.5)	20 (46.5)	1.0	0.02
Age, mean (SD), years	65.1 (8.1)	67.2 (7.3)	63.5 (8.5)	0.05	0.46
Education level above 4-yr, n (%)	19 (25.0)	2 (6.1)	17 (39.5)	0.001	0.38
HPA function parameters					
Morning ACTH (pg/ml)					
Baseline	20.5 (11.7 – 31.3)	20.5 (13.2 – 30.5)	18.0 (10.5 – 32.4)	0.47	0.17
Last	17.8 (11.8 – 25.9)	17.9 (12.3 – 26.6)	17.2 (11.4 – 26.3)	0.76	0.07
Morning plasma cortisol (mcg/dl)					
Baseline	15.8 (4.2)	16.1 (4.1)	15.6 (4.3)	0.66	0.12
Last	15.8 (4.4)	16.5 (5.6)	15.3 (3.2)	0.23	0.27
Morning plasma cortisol/ACTH ratio (mcg/dl *per* pg/ml)					
Baseline	0.81 (0.52 – 1.12)	0.75 (0.52 – 1.07)	0.85 (0.53 – 1.26)	0.45	0.008
Last	0.91 (0.58 – 1.21)	0.85 (0.56 – 1.18)	0.95 (0.62 – 1.22)	0.67	0.002
Morning urinary cortisol/creatinine ratio (mcg/g)					
Baseline	10.5 (7.4 – 16.1)	10.7 (7.5 – 16.3)	9.7 (7.3 –15.9)	0.81	0.001
Last	16.4 (10.7 – 28.0)	21.6 (12.8 – 29.5)	13.6 (8.5 – 24.8)	0.09	0.04
Cognitive scores and trends,					

Median (IQR)					
Baseline MEM score	0.18 (-0.97 to 0.66)	-0.98 (-1.27 to -0.68)	0.59 (0.31 to 0.87)	<0.001	0.68
Baseline EXEC score	-0.01 (-0.87 to 0.69)	-0.89 (-1.10 to -0.68)	0.61 (0.27 to 1.23)	<0.001	0.66
Last MEM score	-0.50 (-1.27 to 0.65)	-1.16 (-1.47 to -0.88)	0.25 (-0.54 to 1.16)	<0.001	0.31
Last EXEC score	-0.29 (-0.96 to 0.79)	-0.96 (-1.25 to -0.54)	0.67 (0.20 to 1.19)	<0.001	0.65
MEM score Trend	-0.22 (-0.71 to 0.28)	-0.12 (-0.49 to 0.28)	-0.31 (-1.10 to 0.30)	0.09	0.04
EXEC score Trend	0.001 (-0.09 to 0.15)	0.005 (-0.12 to 0.16)	-0.01 (-0.09 to 0.14)	0.62	0.003

HPA, hypothalamus-pituitary-adrenal; ACTH, adreno-corticotropic hormone; IQR, interquartile range; MEM, memory dimension composite; EXEC, executive function dimension composite.

On univariate analysis, "Good" performers were tendentially younger (p=0.051, *Cohen's d*=0.46) and had higher education level than "Poor" performers (p=0.001, ϕ=0.38). No differences were found between the two groups regarding female/male ratio, morning ACTH, morning plasma total cortisol, plasma cortisol/ACTH ratio, morning urinary free cortisol and urinary cortisol/creatinine ratio, both at baseline and last follow-up evaluation. Regarding to the two distinct collections time-points, morning plasma cortisol/ACTH ratio did not change significantly over time in both groups ("Poor" 0.75 *vs.* 0.85, p=0.08; "Good" 0.85 *vs.* 0.95, p=0.52), but morning urinary cortisol/creatinine ratio did increased in both groups from baseline to the last assessment ("Poor" 10.7 *vs.* 21.6, p<0.001; "Good" 9.7 *vs.* 13.6, p<0.001). The magnitude of this rise was not different between the two groups ("Poor" vs. "Good" median, 7.2 *vs.* 4.2; p=0.22).

In order to evaluate the HPA stability on each individual HPA axis performance during the follow-up we considered, in each group, participants that remained within the same respective tercile on both determinations for morning plasma cortisol, morning plasma cortisol/ACTH ratio and morning urinary cortisol/creatinine ratio. In the "Poor" group, overall HPA profile consistency was of 39% (13/33), 61% (20/33) and 33% (11/33) for morning plasma cortisol, morning plasma cortisol/ACTH ratio and morning urinary cortisol/creatinine ratio, respectively. In the "Good" group, overall profile consistency was of 44% (19/43), 63% (27/43) and 65% (28/43) for morning plasma cortisol, morning plasma cortisol/ACTH ratio and morning urinary cortisol/creatinine ratio,

respectively. Significant association was found between baseline and last morning plasma cortisol levels in the "Good" (r=0.41, p=0.006) but not in the "Poor" group (r=0.16, p=0.38). Similarly, noteworthy correlations were found between the two collections for morning plasma cortisol/ACTH ratio in both groups ("Poor": r=0.72, p<0.001; "Good": r=0.75, p<0.001). Baseline morning urinary cortisol/creatinine ratio correlated with its following determination only within the "Good" performers group ("Good": r=0.67, p<0.0001; "Poor": r=0.25, p=0.18).

Having this intra-individual HPA axis consistency levels in mind, we pursued to the cognitive association analysis on the following HPA axis metrics: morning plasma cortisol, morning plasma cortisol/ACTH ratio and morning urinary cortisol/creatine ratio. Correlation coefficients weighing cognitive scores and trends with these HPA axis measures are presented in Tables 4.2, 4.3 and 4.4.

Table 4.2. Pearson's correlation coefficient (r) between **Morning Plasma Cortisol** levels and MEM and EXEC scores and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 33)		**"Good" (n = 43)**	
	Baseline Morning Plasma Cortisol	Last Morning Plasma Cortisol	Baseline Morning Plasma Cortisol	Last Morning Plasma Cortisol
Baseline MEM score	0.08	–	0.27	–
Baseline EXEC score	0.01	–	0.23	–
Last MEM score	0.04	-0.16	0.11	-0.14
Last EXEC score	-0.09	-0.05	0.22	0.11
MEM score trend	-0.02	-0.07	-0.05	-0.22
EXEC score trend	-0.18	-0.03	-0.04	-0.09

Table 4.3. Spearman's rank correlation (r_s) between **Morning Plasma Cortisol/ACTH ratio** and MEM and EXEC scores and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 33)		**"Good" (n = 43)**	
	Baseline Morning Pl. Cortisol/ACTH ratio	Last Morning Pl. Cortisol/ACTH ratio	Baseline Morning Pl. Cortisol/ACTH ratio	Last Morning Pl. Cortisol/ACTH ratio
Baseline MEM score	-0.23	–	-0.09	–
Baseline EXEC score	0.08	–	-0.13	–
Last MEM score	-0.14	-0.22	0.007	0.13
Last EXEC score	-0.05	-0.01	-0.14	0.03
MEM score trend	0.02	-0.06	0.06	0.14
EXEC score trend	-0.07	-0.04	-0.13	-0.11

Table 4.4. Spearman's rank correlation (r_s) between **Morning Urinary Cortisol/Creatinine ratio** and MEM and EXEC scores and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 33)		**"Good" (n = 43)**	
	Baseline Morning Ur. Cortisol/Creat. ratio	Last Morning Ur. Cortisol/Creat. ratio	Baseline Morning Ur. Cortisol/Creat. ratio	Last Morning Ur. Cortisol/Creat. ratio
Baseline MEM score	0.15	–	-0.10	–
Baseline EXEC score	0.26	–	-0.37[a]	–
Last MEM score	0.09	-0.19	-0.04	-0.21
Last EXEC score	0.05	0.04	-0.44[b]	-0.50[c]
MEM score trend	-0.01	-0.05	-0.02	-0.24
EXEC score trend	-0.02	0.24	-0.24	-0.24

[a] $p = 0.01$; [b] $p = 0.03$; [c] $p < 0.001$.

In the "Good" performers group, moderately strong negative correlations were found between morning urinary cortisol/creatinine levels and executive composite scores at distinct cognitive assessments (r_s = -0.37 for baseline levels; r_s = -0.44 between baseline urinary levels and last EXEC score; and r_s = -0.50 for the last parallel determinations).

There were no association between baseline or last follow-up morning urinary cortisol/creatinine ratios, morning plasma cortisol or morning plasma cortisol/ACTH ratio with MEM or EXEC scores in "Poor" and "Good" groups.

To explore the association between the observed correlation with EXEC scores and morning urinary cortisol/creatinine ratio levels in the "Good" sub-group, a multiple linear regression analysis for each time-point was performed adjusted for baseline age, gender and education level (Table 4.5 and 4.6).

Table 4.5. Association between observed **Executive Function** scores with Age, Gender, Education Level and **baseline Morning Urinary Cortisol/Creatinine ratio** predicted by multiple linear regression analysis within the **"Good" performer group**, (n = 43), (baseline EXEC score, p <0.001, R^2 = 0.47, adjusted R^2 = 0.42; last EXEC score, p <0.001, R^2 = 0.44, adjusted R^2 = 0.39).

	Baseline EXEC score		**Last EXEC score**	
	β (95% CI)	*p*	β (95% CI)	*p*
Age (years)	-0.04 (-0.06 to -0.02)	0.001	-0.04 (-0.06 to -0.01)	0.003
Gender (Exposure: Male)	-0.20 (-0.54 to 0.14)	0.24	-0.17 (-0.56 to 0.21)	0.37
Education Level (years)	0.04 (-0.01 to 0.08)	0.08	0.05 (-0.001 to 0.09)	0.06
Baseline Morning Urinary Cortisol/Creatinine ratio	-0.44 (-2.32 to 1.44)	0.64	-0.60 (-2.73 to 1.53)	0.57

Table 4.6. Association between observed **Executive Function** scores with Age, Gender, Education Level and **last Morning Urinary Cortisol/Creatinine ratio** predicted by multiple linear regression analysis within the **"Good" performer group**, (n = 43), (last EXEC score, *p* <0.001, R^2 = 0.45, adjusted R^2 = 0.39).

	Last EXEC score	
	β (95% CI)	*p*
Age (years)	-0.04 (-0.06 to -0.01)	0.003
Gender (Exposure: Male)	-0.15 (-0.54 to 0.24)	0.44
Education Level (years)	0.05 (0.001 to 0.09)	0.05
Last Morning Urinary Cortisol/Creatinine	-0.57 (-1.86 to 0.72)	0.38

In these two fully adjusted models, baseline and last morning urinary cortisol/creatinine ratio failed to predict executive cognitive performance both cross-sectionally and over time. In the baseline EXEC score model, baseline morning urinary cortisol/creatinine ratio β coefficient was -0.44 (95%CI, -2.32 to 1.44; *p*=0.64) whereas in the last EXEC score, it was -0.60 (-2.73 to 1.53, *p*=0.57). Last morning urinary cortisol/creatinine ratio was also not associated to the concomitant executive neuro-cognitive performance [β -0.57 (-1.86 to 0.72); *p* = 0.38].

Discussion

In this elder population-based study, steady-state HPA axis measurements were not substantially related to cognitive performance, both cross-sectional and longitudinally.

Adrenal steroids are fundamental for the brain function (Sousa and Almeida 2002). Stress response modulation by the HPA axis is closely linked with the prefrontal cortex, a brain region essential for the working memory and executive function (critical portions of the human cognition) (McEwen and Morrison 2013). Some authors proposed an inverted U-shape relationship between cortisol and overall cognition, whereas too low or too high levels seem to be harmful to the brain processing (Joels 2006). A loss of feed-back control of the ACTH/cortisol secretion that results in elevated mean 24h cortisol levels and the loss of the cortisol circadian *nadir* with an increased levels of overnight cortisol production is also commonly found in the elderly (Veldhuis, Sharma et al. 2013).

Here we have found a moderately strong negative correlation between morning urinary cortisol/creatine levels (a marker of nocturnal HPA activation) and executive composite scores of the "Good" performer group in every time assessed. However, this association was lost upon adjustment for age, gender and education. This may result from the small number of individuals included in this group but also because of the "floor" phenomenon. This effect results from the fact that those individuals already clustered around lower levels of the neuro-cognitive measures will not descend further than this limit and therefore will fail to present any visible response to expected modulators. To date, most studies published have also linked the nocturnal HPA axis hyperactivation to an inferior cognitive performance. In a 4,244 Icelandic cohort (mean age 76-yr), a poorer cognitive functioning across several domains (including executive) was associated to higher evening salivary cortisol levels (Geerlings, Sigurdsson et al. 2015). In the Baltimore Memory Study (participants aged 50 to 70 years) a higher mean diurnal salivary cortisol was related with worse performance in several domains (language, processing speed, eye-hand coordination, executive functioning, verbal memory and learning, and visual memory) (Lee, Glass et al. 2007). Longitudinal studies failed to prove a coherent link between overall higher evening cortisol levels and cognition (Singh-Manoux, Dugravot et al. 2014).

With respect to the other HPA axis metrics (morning plasma cortisol and morning plasma cortisol/ACTH ratio), we did not find association with baseline and last evaluated MEM and EXEC composite scores. Some contradictory results have also been reported in the same Icelandic discussed above, where the same morning cortisol levels that were positively associated with a better executive function were not linked to improved memory performance (Geerlings, Sigurdsson et al. 2015). Regarding early morning HPA axis status, an increase HPA axis activation, demonstrated by an elevated cortisol awakening response slope, has been associated to better working memory and executive performance (Almela, van der Meij et al. 2012). Due to its methodological difficulties' cortisol awakening response analysis is not usually performed in population-based studies and was not included in the present study. Importantly, we observed that morning plasma cortisol levels were not consistent overtime within individuals, especially within "Poor" performers (only 39% stayed in same tercile), and that there was no correlation between baseline and last follow-up determinations in this group (r=0.16, p=0.38). A more stable way of looking for morning HPA activation could be obtained by evaluating morning plasma cortisol/ACTH ratio. In the present study this measure was relatively stable within individuals (correlations of r = 0.72 and 0.75, with 61% and 63% staying within the same tercile in "Poor" and "Good" performers

group, respectively). Despite this constancy, no significant correlation was detected between these measurements and EXEC and MEM scores, therefore failing to observe a putative cognitive beneficial effect for an elevated morning cortisol surge. The literature reveals some controversy on these measures. In a prospective study with 97 elderly men, elevated plasma morning cortisol was actually linked to worse working memory and episodic memory tasks (MacLullich, Deary et al. 2005). Also in a large longitudinal assessment of cognitive functioning, higher levels of average area-under-the-curve diurnal cortisol output was significantly associated with poorer performance in executive, processing speed and visuo-spatial memory domains (Franz, O'Brien et al. 2011).

Intervention trials have tried to explore "acute" cortisol effects on neuro-cognitive tests, some of them reporting enhanced memory consolidation while others observing decreasing performance in declarative memory tasks (Buchanan and Lovallo 2001, Het, Ramlow et al. 2005). A recent meta-analysis presented no evidence for a significant relationship between these "acute "cortisol effects and core executive functions (Shields, Sazma et al. 2016).

Some important limitations of the present study must be specified. First, small sample size may not allow for small differences to be detected. Second, there was a relatively short period of time between the two prospective cognitive evaluations (min-max: 16-22 months), which may have also weakened the ability to detect any subtle time-dependent intra or inter-individual cognitive differences, and therefore underestimating potential associations with HPA axis status. Third, blood and urine sampling done at two separate time-points may be insufficient to reveal the real individual HPA axis set-point which could lead to HPA steady-state category "misclassifications". Very few studies have addressed this intra-individual HPA axis day-to-day patterns. The ones published have done it only with plasma and salivary cortisol level changes, showing time-point variations within-subject ranging from 6 to 103% (Casals, Foj et al. 2011). Intra-individual variability is an important limitation frequently found in many other studies with older adults, making it harder to discover any association between cognitive outcomes and specific HPA axis set-points.

The study main strengths are grounded on the exhaustive cognitive evaluation performed longitudinally with the construction of coherent executive and memory cognitive domain composites and in the two-point HPA axis assessment (steady-state morning and nocturnal cortisol secretion) based in simple, non-invasive, HPA hormone determinations.

In conclusion, cognitive performance in this healthy ageing cohort was not independently associated with higher nocturnal or morning activation of HPA axis, somewhat contradicting the proposed thesis of "glucocorticoid cascade" and the associated cognitive decline (Muller, Lucassen

et al. 2001). A better understanding of the link between HPA axis and adrenal steroids during the brain ageing process is warranted with future studies compelled to address better the role of time-window exposure, intra-individually variations and "chronic" *versus* "acute" HPA activation.

5. Longitudinal analysis of HPT axis influence on the cognitive performance of a healthy ageing cohort

Abstract

Background: Overt thyroid dysfunction is associated to cognitive disturbances. However, conflicting information is available on the relation between TSH or thyroid hormones and cognitive function in older euthyroid individuals. Our purpose was to evaluate, longitudinally, the association of thyroid function with cognitive performance in a healthy older adults' cohort.

Methods: Seventy-seven individuals over 55 years-old with no cognitive impairment, clustered as healthy "Poor" and "Good" cognitive performers, were followed for an average of 18 months (min-max, 16-22 months). Baseline and last visit TSH, FT_4 and TT_3 levels were related, longitudinally, with cognitive (memory and general/executive) composite scores and temporal trends.

Results: No differences were found in thyroid function, iodine status and thyroid volume between the two groups. In an unadjusted analysis, higher TSH levels at baseline were associated to better baseline memory composite scores in the "Poor" cognitive performers group (r=0.43, p=0.01). Within the same group, last visit TSH was positively correlated to concomitant executive composite scores (r=0.36, p=0.04). Baseline and last-visit FT_4 levels were inversely associated to last evaluation executive functioning scores, also in the "Poor" performers group (r= -0.42, p=0.02; r= -0.55, p=<0.001; respectively). In these same group, lower baseline FT_4 levels were associated to better baseline memory performance scores (r= -0.36, p=0.04). Longitudinal memory and executive function trend analysis failed to identify any connection to thyroid function measurements, except for last-visit FT_4 levels that was negatively correlated with executive score trend (r= -0.46, p=0.007). No significant correlations were found between thyroid function parameters and memory or executive composite scores in the "Good" performers group. The associations in the "Poor" performance group were lost after adjustments for age, gender and education level.

Conclusion: In this healthy iodine-deficient older population with no cognitive impairment, thyroid function is not longitudinally associated with memory and executive cognitive composite scores.

Introduction

Thyroid hormones (TH) are crucial elements for early human brain development (Prezioso, Giannini et al. 2018). Adult-onset thyroid dysfunction is also known to cause reversible overall brain perturbations (Gan and Pearce 2012). Hypothyroid patients have been classically described as presenting a TH-dependent reversible impairment in many cognitive domains, such as attention, learning and visual-spatial abilities (Osterweil, Syndulko et al. 1992, Constant, Adam et al. 2005). Thyrotoxicosis patients are also recognized to perform worse in some neurocognitive executive tasks (Vogel, Elberling et al. 2007, Kumar, Rana et al. 2019). As for euthyroid individuals, the question continues open on the relation between TH within the euthyroid range and cognition.

Populational studies showed that normal ageing is related to a small increase in TSH levels, which results into an increased high-normal TSH range in older subjects (Surks and Hollowell 2007). Data on FT_4 distribution has been more contradictory, with some reports describing an upward curve while others concluding for no clear age-related changes (Hoogendoorn, Hermus et al. 2006, Bremner, Feddema et al. 2012). Little is known about the influence of these hormone adjustments in cognitive performance during healthy ageing. Some cross-sectional and prospective studies have linked lower TSH and higher FT_4 serum concentrations to worse cognitive performance (Hogervorst, Huppert et al. 2008, Livner, Wahlin et al. 2009). Recently, however, two large longitudinal studies with older individuals found no such correlation between baseline "normal" TSH or FT_4 levels and neurocognitive tests (Tan, Beiser et al. 2008, Samuels, Kaimal et al. 2016).

These apparent contradictory statements and some lack of representative studies assessing the full longitudinal HPT axis/cognition relationship within healthy ageing populations prompt us to conduct the current study. Here, the objective was to evaluate, prospectively, the association between cognitive performance and HPT axis hormones based in an older Portuguese community-dwelling population.

Methods

Subjects

Sample characterization has been presented in chapter 3.

For analysis purposes, subjects with prior history of thyroid surgery or chronic thyroid dysfunction, cerebrovascular disorders, dementia, or those who were on levothyroxine were excluded. Individuals who presented TSH levels above 10mUI/ml or below 0.1mUI/ml and who that did not have both plasma TSH or FT_4 concentration data available at baseline and at the last follow-up

evaluation (min-max: 16-22 months) were also excluded. The final sample for consideration for the full analysis included 77 participants (Figure 5.1).

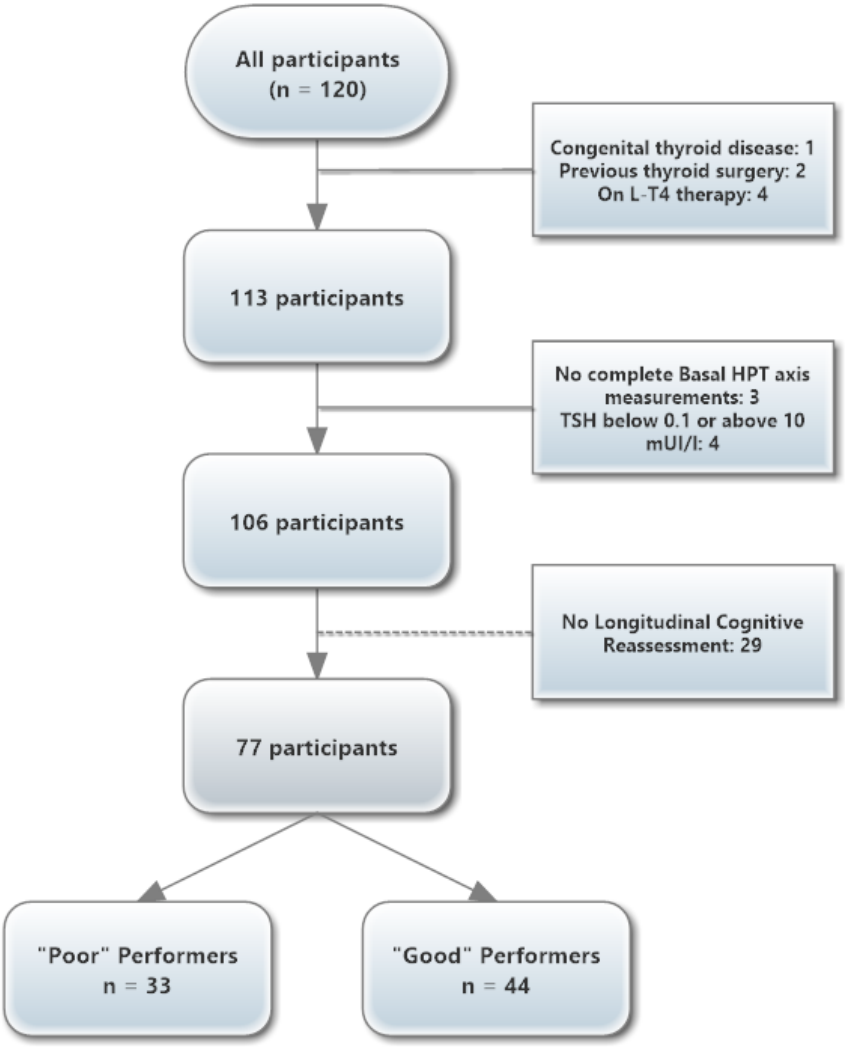

Figure 5.1. Overview of the participant flowchart. L-T_4, levothyroxine; HPT, hypothalamus-pituitary-thyroid.

Analytical methods

All subjects underwent fasting for morning blood collection. Blood samples were collected, centrifuged and stored at -20°C until hormone levels determination. First morning urinary samples were collected and stored accordantly. Urinary iodine content was assessed spectrophotometrically using a modification of the *Sandell–Kolthoff* reaction, with an initial ammonium persulfate digestion (Pino, Fang et al. 1998). A standard curve was obtained for each batch of digested samples and the final measurements were performed in a laboratory that participates in the ongoing

international CDC-Atlanta inter-calibration program Ensuring the Quality of Urinary Iodine Procedures (EQUIP) for the determination of iodine in human urine samples with over 99% success (CDC 2017). Plasma concentrations of TSH, FT_4 and T_3 levels were measured by Siemens Dimension Vista® System chemiluminescent immunometric assay. The TSH/FT_4 ratio (mUI/ml *per* ng/dl) was used as a potential marker of the steady-state HPT status. Antibodies anti-TPO (antithyroid peroxidase) were measured by Immulite® 2000 System chemiluminescent immunometric assay, with levels less than 35 IU/ml indicating normal serum anti-TPO concentration.

Thyroid volume

Thyroid volume (Tvol) was assessed accordingly to published WHO standards by a trained medical doctor (ACC) with experience on cervical ultrasound (WHO, UNICEF et al. 2007). A portable ultrasound (Mindray, Shenzhen, P. R. China) equipped with a 60 mm 5-10 MHz linear transducer was used for measurements. Subjects were examined in a seated position with extended cervical spine. Maximum perpendicular depth (AP) and width (ML) were measured with electronic calipers on a transverse image of the largest diameter. The maximum lobe length (CC) was measured on a longitudinal image. The transducer was kept perpendicular to the skin. Nodules and/or cystic areas were included in the volume determination. The thyroid gland volume was calculated without isthmus by adding the volume of the right and left lobes, each calculated as follows: Thyroid volume lobe = AP diameter × ML diameter × CC diameter × 0.479 (Brunn, Block et al. 1981).

Cognitive assessment

As specified in chapter 3.

Statistical analysis

All data are presented as the mean (median), SD (inter-quartile-range, IQR) for normally (non-normally) distributed data. All continuous variables were checked for normality using the Shapiro-Wilk normality test. Paired and unpaired *t*-test and Mann-Whitney U-test were used to compare continuous variables between the two groups, as appropriate. Wilcoxon matched pairs signed rank test was performed to compare paired variables that failed normality tests. Fisher's exact test was used for categorical variables. Univariate analysis with Pearson correlation coefficient or Spearman's rank correlation was performed, as appropriate, to assess linear relationship between different HPT axis status measurements and the different cognitive domain scores (MEM and

EXEC) and its temporal trends, stratified by cognitive group. A multiple linear regression analysis was conducted to explore the prediction of Memory/Executive function scores and trends by any HPT axis metrics found relevant in univariate assessment but adjusted to age, gender, baseline cognitive group and education level. Effect size estimates were calculated with Cohen's d and η^2 for continuous variables with parametric and non-parametric comparisons, respectively; r for Wilcoxon matched paired test; ϕ coefficient for Fisher's exact test; and R^2 and adjusted R^2 for regression analysis.

All analyses were tested at the 0.05 level of significance and performed using IBM SPSS Statistics, v.21 (IBM, New York USA) and GraphPad Prism, v.6.00 (GraphPad Software, La Jolla California USA). Effect size estimates were evaluated by Cohen's published benchmark classes (small, medium and large) with the following proposed cut-points: 0.2, 0.5 and 0.8 for Cohen's d; 0.01, 0.06, 0.14 for η^2; 0.1; 0.3 and 0.5 for r and ϕ coefficient; and 0.02, 0.13, 0.26 for R^2 (Cohen 1988).

Results

The final sample included 77 individuals with a mean age of 66.0-yr (SD 8.2-yr) and women ratio of 0.42 (n=32). Total study population characteristics are presented in Table 5.1.

Table 5.1. Study population characteristics by cognitive performance group.

Variable	Total (n = 77)	"Poor" (n = 33)	"Good" (n = 44)	*p*	Effect-size
Socio-demographic features					
Sex					
Female, n (%)	32 (41.6)	15 (45.5)	17 (38.6)	0.64	0.07
Age, mean (SD), years	66.0 (8.2)	68.0 (7.2)	64.6 (8.7)	0.07	0.42
Education level above 4-yr, n (%)	22 (29.0)	2 (6.1)	20 (45.5)	<0.001	0.43
Urinary iodine concentration (mcg/l)* (IQR)	54.9 (37.2 – 87.6)	51.9 (31.7 – 93.4)	56.9 (39.2 – 85.9)	0.64	0.02
Thyroid volume** (cm^3) (IQR)	9.6 (7.3 – 13.8)	9.5 (6.8 – 14.8)	9.6 (7.6 – 11.6)	0.73	0.33

HPT parameters					
TSH (mUI/ml), median (IQR)					
Baseline	1.56 (1.15 – 2.15)	1.95 (1.15 – 2.59)	1.40 (1.13 – 1.95)	0.15	0.02
Last	1.64 (1.14 – 2.26)	1.71 (1.14 – 2.60)	1.64 (1.06 – 1.96)	0.42	0.006
FT_4 (ng/dl), mean (SD)					
Baseline	0.98 (0.13)	0.95 (0.16)	1.00 (0.10)	0.13	0.39
Last	0.99 (0.14)	0.98 (0.15)	1.00 (0.13)	0.45	0.14
TSH-FT_4 ratio (mUI/l *per* ng/dl), median (IQR)					
Baseline	1.6 (1.2 – 2.4)	2.1 (0.9 – 3.0)	1.5 (1.2 – 2.1)	0.09	0.03
Last	1.6 (1.1 – 2.4)	1.7 (1.1 – 2.9)	1.6 (1.1 – 2.1)	0.36	0.008
TT_3 (ng/ml)					
Baseline	1.12 (0.19)	1.14 (0.19)	1.10 (0.19)	0.38	0.21
Atb Anti-TPO positivity, n (%)§	3 (4.8)	3 (13.0)	0 (0)	0.05	0.29
Thyroid with heterogeneous echogenicity, n (%)**	12 (26.7)	5 (25.0)	7 (28.0)	1.0	0.03
Atb Anti-TPO positivity OR Thyroid heterogeneous echogenicity, n (%)#	12 (16.2)	5 (16.7)	7 (15.9)	1.0	0.05
Cognitive scores and trends, Median (IQR)					
MEM score					
Baseline	0.18 (-0.96 to 0.68)	-0.97 (-1.27 to -0.60)	0.59 (0.31 to 0.87)	<0.001	0.67
Last	-0.74 (-1.20 to 0.66)	-1.15 (-1.42 to -0.83)	0.29 (-0.82 to 1.15)	<0.001	0.26
EXEC score					

Baseline	-0.004 (-0.83 to 0.69)	-0.87 (-1.18 to -0.60)	0.60 (0.25 to 1.19)	<0.001	0.66
Last	-0.29 (-0.94 to 0.78)	-0.96 (-1.25 to -0.53)	0.65 (0.19 to 1.19)	<0.001	0.65
MEM score Trend	-0.20 (-0.71 to 0.29)	-0.11 (-0.43 to 0.28)	-0.31 (-1.30 to 0.32)	0.07	0.04
EXEC score Trend	-0.003 (-0.12 to 0.15)	-0.003 (-0.17 to 0.16)	-0.02 (-0.09 to 0.14)	0.43	0.008

Abd, antibodies; Anti-TPO, antithyroid peroxidase; EXEC, executive function dimension composite; FT_4, free thyroxine; HPT, hypothalamus-pituitary-thyroid; IQR, interquartile range; MEM, memory dimension composite; SD, standard deviation; TSH, thyrotropin; TT_3, total triiodothyronine.

* n=72; ** n=45; § n=62; # n=74.

On univariate analysis, "Good" performers were tendentially younger (p=0.07, *Cohen's d*=0.42) and had higher education level (p=0.001, ϕ=0.43) than "Poor" performers. No differences were found between the two groups regarding female/male ratio, thyroid volume, urinary iodine content and ultra-sound or immunologic evidence of chronic lymphocytic thyroiditis (*i.e.* presence of thyroid with heterogeneous echogenicity or positive anti-TPO antibodies). TSH, FT_4 and TSH-FT_4 ratio levels, both at baseline and last follow-up evaluation, were also similar between groups.

Regarding HPT status "stability" between base line and last-evaluation measurements, TSH concentrations correlated well overtime (r_s=0.82, p<0.001), just like FT_4 levels (r=0.67, p<0.001). In the "Poor" group, overall HPT profile consistency with conservation within the same tercile through both evaluations was of 79% (26/33), 67% (22/33) and 76% (25/33) for TSH, FT_4 and TSH-FT_4 ratio, respectively. In the "Good" group, overall profile consistency was of 68% (30/44), 64% (28/44) and 66% (29/44) for TSH, FT_4 and TSH-FT_4 ratio, respectively.

Correlation coefficients weighing cognitive scores and trends with HPT axis hormones are presented in Tables 5.2, 5.3, 5.4 and 5.5.

Table 5.2. Spearman's rank correlation (r_s) between **TSH levels** and **MEM and EXEC scores** and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 33)		**"Good" (n = 44)**	
	Baseline TSH	Last TSH	Baseline TSH	Last TSH
Baseline MEM score	**0.43***	–	-0.22	–
Baseline EXEC score	0.25	–	0.03	–
Last MEM score	-0.24	0.14	0.11	-0.14
Last EXEC score	**0.34#**	**0.36§**	0.03	-0.24
MEM score trend	-0.12	-0.12	0.21	-0.003
EXEC score trend	0.19	0.26	0.09	-0.05

* p=0.01; # p=0.05; § p=0.04.

Table 5.3. Pearson's correlation coefficient (r) between **free T_4** levels and **MEM and EXEC scores** and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 33)		**"Good" (n = 44)**	
	Baseline FT4	Last FT4	Baseline FT4	Last FT4
Baseline MEM score	**-0.36***	–	-0.23	–
Baseline EXEC score	**-0.33#**	–	-0.12	–
Last MEM score	-0.16	-0.08	-0.08	-0.08
Last EXEC score	**-0.42§**	**-0.55****	-0.05	-0.25
MEM score trend	0.11	0.23	0.05	0.14
EXEC score trend	-0.29	**-0.46##**	0.21	0.12

* p=0.04; # p= 0.06; § p=0.02; ** p<0.001; ## p=0.007.

Table 5.4. Spearman's rank correlation (r_s) between **TSH/FT_4 ratio** and **MEM and EXEC scores** and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 33)		**"Good" (n = 44)**	
	Baseline TSH/FT_4 ratio	Last TSH/FT_4 ratio	Baseline TSH/FT_4 ratio	Last TSH/FT_4 ratio
Baseline MEM score	**0.47***	–	-0.18	–
Baseline EXEC score	0.29	–	0.03	–
Last MEM score	0.22	0.17	0.10	-0.11
Last EXEC score	**0.40#**	**0.42#**	0.01	-0.14
MEM score trend	-0.15	-0.11	0.17	-0.07
EXEC score trend	0.27	**0.33§**	0.01	0.01

* p =0.006; # p=0.02; § p=0.06.

Table 5.5. Spearman's rank correlation (r_s) between **total T_3 levels** and **MEM and EXEC scores** and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 33)	**"Good" (n = 44)**
	Baseline T_3	Baseline T_3
Baseline MEM score	0.25	0.09
Baseline EXEC score	0.27	0.24
Last MEM score	0.20	0.08
Last EXEC score	0.14	0.23
MEM score trend	-0.07	0.01
EXEC score trend	0.01	0.09

Significant correlations were only identified within the "Poor" performers group. Among these individuals, baseline memory composite scores were moderately strong correlated to baseline TSH levels and TSH-FT_4 ratio (r_s=0.43, p=0.01; r_s=0.47, p=0.006, respectively). In the same cognitive group, a moderate strong negative correlation was found between baseline memory composite score and baseline FT_4 levels (r= -0.36, p=0.04). Last visit executive scores were also correlated positively to baseline and last TSH levels (r_s=0.34, p=0.05; r_s=0.36, p=0.04, respectively), as well as to baseline and last TSH-FT_4 ratio (r_s=0.40, p=0.02; r_s=0.42, p=0.02, respectively). Last obtained executive scores in "Poor" performers group were inversely correlated, in a moderate/strong way, to FT_4 levels (r= -0.42, p=0.02; r= -0.55, p<0.001, respectively for baseline and last visit FT_4 determinations). T_3 levels did not correlate to any cognitive composite score in both cognitive groups. Memory composite trend overtime was not correlated to any of the HPT axis measurements, but executive composite score trend was inversely correlated, in a moderate/strong way, to the final visit FT_4 determination (r= -0.46, p=0.007), again only in the "Poor" performer group.

After adjustment for age, gender and education level all these significant interactions between cognitive composite scores and TSH or FT_4 levels in the "Poor" performers group were lost (Table 5.6 to 5.14).

Table 5.6. Association between observed **baseline Memory Function** scores with Age, Gender, Education Level and **baseline TSH levels** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 33), (baseline MEM score, p =0.10, R^2 = 0.24, adjusted R^2 = 0.13).

	Baseline MEM score	
	β (95% CI)	p
Age (years)	-0.20 (-0.04 to 0.01)	0.27
Gender (Exposure: Male)	-0.02 (-0.34 to 0.29)	0.91
Education Level (years)	0.05 (-0.03 to 0.17)	0.16
Baseline TSH levels (mUI/ml)	0.21 (-0.06 to 0.24)	0.22

Table 5.7. Association between observed **baseline Memory Function** scores with Age, Gender, Education Level and **baseline free T_4 levels** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 33), (baseline MEM score, p =0.11, R^2 = 0.23, adjusted R^2 = 0.12).

	Baseline MEM score	
	β (95% CI)	p
Age (years)	-0.17 (-0.04 to 0.02)	0.42
Gender (Exposure: Male)	-0.01 (-0.32 to 0.31)	0.96
Education Level (years)	0.25 (-0.04 to 0.17)	0.18
Baseline T_4 levels (ng/dl)	-0.21 (-1.6 to 0.51)	0.29

Table 5.8. Association between observed **baseline Memory Function** scores with Age, Gender, Education Level and **baseline TSH/FT4 ratio** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 33), (baseline MEM score, p =0.10, R^2 = 0.24, adjusted R^2 = 0.13).

	Baseline MEM score	
	β (95% CI)	*p*
Age (years)	-0.18 (-0.04 to 0.01)	0.36
Gender (Exposure: Male)	-0.002 (-0.32 to 0.31)	0.99
Education Level (years)	0.26 (-0.03 to 0.17)	0.17
Baseline T_4 levels (ng/dl)	0.23 (-0.05 to 0.21)	0.22

Table 5.9. Association between observed **Executive Function** scores with Age, Gender, Education Level and **baseline TSH levels** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 33), (baseline EXEC score, p <0.001, R^2 = 0.54, adjusted R^2 = 0.48; last EXEC score, p <0.001, R^2 = 0.54, adjusted R^2 = 0.46).

	Baseline EXEC score		**Last EXEC score**	
	β (95% CI)	*p*	β (95% CI)	*p*
Age (years)	-0.13 (-0.02 to 0.01)	0.37	-0.16 (-0.03 to 0.01)	0.26
Gender (Exposure: Male)	0.03 (-0.20 to 0.25)	0.83	0.19 (-0.09 to 0.47)	0.18
Education Level (years)	0.68 (0.10 to 0.25)	<0.001	0.66 (0.11 to 0.30)	<0.001
Baseline TSH levels (mUI/ml)	0.07 (-0.08 to 0.14)	0.61	0.11 (-0.08 to 0.19)	0.42

Table 5.10. Association between observed **Executive Function** scores with Age, Gender, Education Level and **baseline free T_4 levels** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 33), (baseline EXEC score, p <0.001, R^2 = 0.55, adjusted R^2 = 0.49; last EXEC score, p <0.001, R^2 = 0.56, adjusted R^2 = 0.50).

	Baseline EXEC score		**Last EXEC score**	
	β (95% CI)	*p*	β (95% CI)	*p*
Age (years)	-0.09 (-0.02 to 0.01)	0.56	-0.08 (-0.03 to 0.02)	0.63
Gender (Exposure: Male)	0.04 (-0.20 to 0.26)	0.78	0.21 (-0.06 to 0.49)	0.12
Education Level (years)	0.67 (0.10 to 0.25)	<0.001	0.65 (0.11 to 0.29)	<0.001
Baseline T_4 levels (ng/dl)	-0.12 (-1.1 to 0.47)	0.44	-0.25 (-1.2 to 0.17)	0.11

Table 5.11. Association between observed **Executive Function** scores with Age, Gender, Education Level and **baseline TSH/FT_4 ratio** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 33), (baseline EXEC score, p <0.001, R^2 = 0.54, adjusted R^2 = 0.48; last EXEC score, p <0.001, R^2 = 0.53, adjusted R^2 = 0.46).

	Baseline EXEC score		**Last EXEC score**	
	β (95% CI)	*p*	β (95% CI)	*p*
Age (years)	-0.13 (-0.02 to 0.01)	0.39	-0.14 (-0.03 to 0.01)	0.34
Gender (Exposure: Male)	0.03 (-0.20 to 0.26)	0.82	0.20 (-0.08 to 0.49)	0.16
Education Level (years)	0.68 (0.10 to 0.25)	<0.001	0.66 (0.11 to 0.30)	<0.001
TSH/FT4 ratio (mUI/ml *per* ng/dl)	0.05 (-0.08 to 0.11)	0.72	0.14 (-0.06 to 0.17)	0.34

Table 5.12. Association between observed **last Executive Function** scores with Age, Gender, Education Level and **last TSH levels** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 33), (last EXEC score, p <0.001, R^2 = 0.54, adjusted R^2 = 0.49).

	Last EXEC score	
	β (95% CI)	p
Age (years)	-0.14 (-0.06 to 0.01)	0.30
Gender (Exposure: Male)	0.21 (-0.54 to 0.24)	0.14
Education Level (years)	0.64 (0.001 to 0.09)	<0.001
Last TSH (mUI/ml)	0.18 (-1.9 to 0.72)	0.20

Table 5.13. Association between observed **last Executive Function** scores with Age, Gender, Education Level and **last free T_4 levels** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 33), (last EXEC score, p <0.001, R^2 = 0.56, adjusted R^2 = 0.50).

	Last EXEC score	
	β (95% CI)	p
Age (years)	-0.14 (-0.03 to 0.01)	0.33
Gender (Exposure: Male)	0.18 (-0.09 to 0.45)	0.19
Education Level (years)	0.56 (0.08 to 0.27)	0.001
Last T_4 (ng/dl)	-0.25 (-1.9 to 0.19)	0.10

Table 5.14. Association between observed **last Executive Function** scores with Age, Gender, Education Level and **last TSH/FT_4 ratio** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 33), (last EXEC score, p <0.001, R^2 = 0.54, adjusted R^2 = 0.48).

	Last EXEC score	
	β (95% CI)	*p*
Age (years)	-0.14 (-0.03 to 0.01)	0.33
Gender (Exposure: Male)	0.21 (-0.07 to 0.49)	0.14
Education Level (years)	0.63 (0.11 to 0.29)	<0.001
Last TSH/FT4 ratio (mUI/ml *per* ng/dl)	0.18 (-0.05 to 0.20)	0.22

Discussion

In this elder population-based study we have found that TSH, FT_4 and TT_3 levels, when adjusted to relevant covariates, were not substantially related to overall cognitive performance, both cross-sectional and longitudinally.

Thyroid hormones are critical for brain development and metabolism. Iodine deficiency is a known feature associated to thyroid dysfunction and if present during early stages of brain maturation are associated with poor cognitive development (Zimmermann and Boelaert 2015). Our older cohort displayed median urinary iodine concentrations in the range of mild iodine deficiency (median UIC between 50-100mcg/l), with no difference between the two cognitive groups (WHO, UNICEF et al. 2007), and in accordance with data in other age groups from the same geographic region (Costeira, Oliveira et al. 2010). Nevertheless, thyroid volume (many times used as a long-term marker of iodine deficiency) showed no increased rate of goiter in this population, probably revealing a rather more "recent" iodine deprived status (data not shown). Alternatively, the iodine insufficiency may be not sufficiently severe to lead thyroid enlargement. Nothing is known about the consequences of such a late-acquired iodine deficiency on the cognitive functions of adult individuals.

In iodine-sufficient populations, the reports on the HTP axis connection to cognitive outcomes during healthy ageing are conflicting. Our cross-sectional unadjusted evaluation found that "normal" TSH levels within "Poor" cognitive performers were positively correlated to memory and, particularly, to executive composite scores. This finding is in accordance with several other observational studies. *Whalin et al.*, evaluating 200 non-demented persons aged 75 to 96 years, found a positively related association between TSH and episodic memory performance, even after adjustments to age, level of education and depressive mood symptoms (Wahlin, Wahlin et al. 1998). More notably, in a prospective analysis of the NHANES III cohort, *Beydoun et al.* described that, within the older group (60 to 90-years of age), higher TSH was associated to better memory and executive functions (Beydoun, Beydoun et al. 2012). Yet most large prospective studies failed to identify such effects. *Volpato et al.* followed 464 healthy older women for 3 years and reported no link between baseline TSH and cognitive function (MMSE) after adjustments to age, education level and other covariates (Volpato, Guralnik et al. 2002). And in a cohort of 539 community-dwelling euthyroid men older than 65-years of age with a mean follow-up of 6-years, *Samuels et al.* reported that cognitive function (evaluated by several neurocognitive tests) was also not related to TSH status (Samuels, Kaimal et al. 2016).

In the present study, FT_4 was negatively correlated to both memory and executive composite scores, again only in the "Poor" performers group and in the unadjusted model. TH connection to cognitive performance in older cohorts has been contradictory in the literature. In a large study (n=3,401) with a mean 6-years follow-up, "normal" high FT_4 levels were associated to an increased risk of dementia (Yeap, Alfonso et al. 2012). *Hogervorst et al.* also recognized that higher FT_4 was associated to worse global cognitive scores in a prospective cohort of 964 older individuals (Hogervorst, Huppert et al. 2008). But some opposite data was observed on younger and smaller cohort studies. *Prinz et al.* reported a positive association between T_4 and cognitive function in 44 men older than 60-years (Prinz, Scanlan et al. 1999). More recently, evaluating near 1,900 individuals (mean age 47-years), *Beyound et al.* found that higher FT_4 was associated to better visuospatial and memory tasks, but this time more expressive in women (Beydoun, Beydoun et al. 2013). Less-biased studies with larger cohorts and longer follow-up time have concluded for a rather neutral effect for, within reference range, FT_4 levels and cognitive performance in older persons (Gussekloo, van Exel et al. 2004, Booth, Deary et al. 2013, Samuels, Kaimal et al. 2016). A meta-analysis including about 25,000 individuals revealed that, despite relevant heterogeneities among publications, higher FT_4 and below-reference TSH values were both associated to an overall increased risk of dementia (Wu, Pei et al. 2016).

Very few longitudinal studies examined correlations between cognitive and thyroid function evolution overtime. Here we observed that, within the "Poor" performers group, last visit FT_4 levels correlate negatively with the memory score trend, which is lost after adjustments to relevant covariates. Similarly, at least 3 reports did not find associations between age-related cognitive decline and TSH or FT_4 levels after the necessary adjustment to confounders (Gussekloo, van Exel et al. 2004, Booth, Deary et al. 2013, Samuels, Kolobova et al. 2016). By contrary, other authors have encountered additional decline in global cognition tasks within individuals with lower TSH and higher FT_4 levels; with a third reporting that low levels of FT_4 were indeed strong predictors of cognitive decline in older men even after adjusting to age, level of education and other covariates (Volpato, Guralnik et al. 2002, Hogervorst, Huppert et al. 2008, Moon, Park et al. 2014).

Importantly, in the present study only the "Poor" performers group presented unadjusted associations between TSH, FT_4 and memory/executive composite scores. This apparent susceptibility to HPT axis variations in the less preserved cognitive (within normality) individuals is intriguing. On brain functional imaging, mild hypothyroid patients (*i.e.* TSH levels slightly above reference range but with FT_4 within "normal" intervals) present reversible decreased activity and

connectivity within brain areas linked to memory and executive functions, such as anterior cingulate cortex, hippocampus and right frontoparietal network (Bauer, Silverman et al. 2009, Singh, Kumar et al. 2015). Also patients with excess TH may present impaired brain connectivity of the frontoparietal network (Kumar, Rana et al. 2019). It is challenging to hypothesize that individuals in the "Poor" performance group, being less cognitive preserved than those in the "Good" performers group, may display less cognitive reserve and be more vulnerable to any negative influence. Therefore, when subjected to transient periods of mild hypo or hyperthyroidism, as recognized by a small elevation of TSH or decrease in FT_4 levels, respectively, they will tend to fall back and reveal more clearly their cognitive gap.

Some important limitations are present in this study. First, due to the small number of participants included with full longitudinal assessment it is possible that the sensitivity to detect important differences between the two distinct cognitive groups is compromised. Second, there was a relatively short time between the two cognitive evaluations (min-max: 16-22 months), which may have also impaired the ability to detect any intra or inter-individual cognitive time-dependent differences, underestimating potential associations with HPT axis status measurements. Third, one blood sample at two distinct time-points may not reveal true intra-individual HPT axis set-point and therefore misclassify their "thyroid biography" status (Franceschi, Ostan et al. 2019). This limitation is frequently found in almost all other longitudinal studies with older adults, making it harder to discover any associations possibly due to the intra-individual and overtime variability found in these cohorts.

One of the main strengths of this study is the longitudinally two-point HPT axis assessment. With this approach we have observed that between 64 to 80% of participants stayed among the same TSH and FT_4 tercile overtime. Data about this longitudinal HTP axis performance is not usually collected and reported in neurocognitive studies. Most have explored associations between cognitive performances and hormone values collected years before, without confirming its hormone axis "stability" and evolution, which, in part, may invalidate the interpretation of some observations. Other major strengths are grounded on the exhaustive cognitive evaluation performed longitudinally with the construction of coherent executive and memory cognitive domain composites assessment. In conclusion, despite some relevant univariate negative interactions between FT_4 levels and executive performance in the "Poor" performers group, both cross-sectionally and prospectively,

cognitive performance in this overall healthy ageing cohort was not independently associated with any HPT axis hormone measurements. A better understanding between HPT axis age-related adjustments and normal brain ageing process is necessary. Future relevant studies addressing the role of time-window exposure, intra-individually variations and "chronic" *versus* "acute" response to TH exposure, are warranted.

6. Longitudinal analysis of GH/IGF-1 axis influence on the cognitive performance of a healthy ageing cohort

Abstract

Background: Conflicting information is available on the relation between GH or IGF-1 and cognitive performance over time in older healthy individuals. Our purpose was to evaluate, longitudinally, the association of parameters of the GH/IGF-1 axis with cognitive performance in a community-dwelling older adults' cohort.

Methods: Fifty-six individuals over 55 years-old with no cognitive impairment, clustered as healthy "Poor" and "Good" cognitive performers, were followed for an average of 18 months. Baseline and last visit fasting GH and IGF-1, as well as post-exercise GH response, were related, longitudinally, with cognitive (memory and executive) composite scores and temporal trends.

Results: Both groups were similar with respect to gender, age, body mass index and body fat mass. "Good" performers were younger and presented higher last visit IGF-1 levels. In multi-variable linear regression models adjusted for gender, age and education level, "Poor" cognitive performers presenting higher executive score trends were associated to lower baseline IGF-1 levels and less decline in IGF-1 (-0.003 per ng/ml increase, 95%CI -0.005 to -0.001, p=0.02; and 0.004 per ng/ml increase, 95%CI 0.001 to 0.007, p=0.01; respectively). In the "Good" cognitive performers, IGF-1 temporal trajectories were linked to memory performance score at the last visit (-0.008 per ng/ml increase, 95%CI -0.02 to <0.001, p=0.05). After the same relevant covariate adjustments, in this group, lower post-exercise GH levels were associated to higher last executive cognitive performance and to less executive abilities decline (-0.14 per ng/ml increase, 95%CI -0.25 to -0.02, p=0.02; and -0.05 per ng/ml increase, 95%CI -0.09 to -0.02, p=0.006; respectively).

Conclusion: In this healthy older population with no cognitive impairment, low baseline levels of IGF-1 are associated to better executive functioning in less preserved cognitive individuals and steep declining IGF-1 age-related trajectories seems to be longitudinally associated with worse executive cognitive trends. In the "Good" performers, post-exercise GH levels are negatively related to executive performance overtime.

Introduction

Growth hormone (GH) and its main mediator, insulin-growth factor 1 (IGF-1) are key regulators of human somatic growth. Early onset GH deficiency syndromes are known to present major cognitive impairment, while late onset GH-deficient individuals are less prone to brain dysfunction (Abbott, Rotnem et al. 1982, Arwert, Deijen et al. 2005). Mixed evidence has been provided from healthy cohort studies. While some authors report better cognitive performances in individuals with lower IGF-1 levels, others found just the opposite or no effect (Kalmijn, Janssen et al. 2000, Licht, van Turenhout et al. 2014, Perice, Barzilai et al. 2016). More recently some reports proposed an inverse U-shape relationship between IGF-1 levels and cognitive performance, suggesting that intermediate levels of IGF-1 are associated to better cognitive functioning (Wennberg, Hagen et al. 2018). Very few studies explored GH enhancing strategies in healthy older adults and cognitive outcomes. Several studies administering recombinant GH, IGF-1 or GH-stimulating diets did not result in better cognitive results (Papadakis, Grady et al. 1996, Friedlander, Butterfield et al. 2001, Arwert, Deijen et al. 2003). However, two randomized control trials have more recently reported improvement in executive functioning, particularly working memory, when GHRH analogue was administered to healthy older participants (Vitiello, Moe et al. 2006, Baker, Barsness et al. 2012). Populational studies have shown that normal ageing is related to a decrease in overall GH secretion and to a steady decrease in IGF-1 levels (Veldhuis 2013). Despite some objective metabolic consequences, currently it is not known if this adaptative change in the GH/IGF-1 axis (defined by some as *somatopause*) has any direct effect over age-related cognitive decline.

All these apparent contradictory statements and some lack of representative studies assessing the full longitudinal GH/IGF-1 axis/cognition relationship within healthy ageing populations prompt us to conduct the current study. Here, the objective was to evaluate, prospectively, the association between cognitive performance and GH/IGF-1 hormones in an older Portuguese community-dwelling population.

Methods

Subjects

Sample characterization has been presented in chapter 3.

For analysis purposes, subjects with of diabetes *mellitus*, renal failure, cerebrovascular disorders or dementia were further excluded. Individuals who did not have both plasma fasting GH or IGF-1

concentration and neurocognitive data available at baseline and last follow-up evaluation (min-max: 16-22 months) were also excluded. The final sample for consideration for the full analysis was of 56 participants, with 49 completing the GH exercise stress protocol (Figure 1).

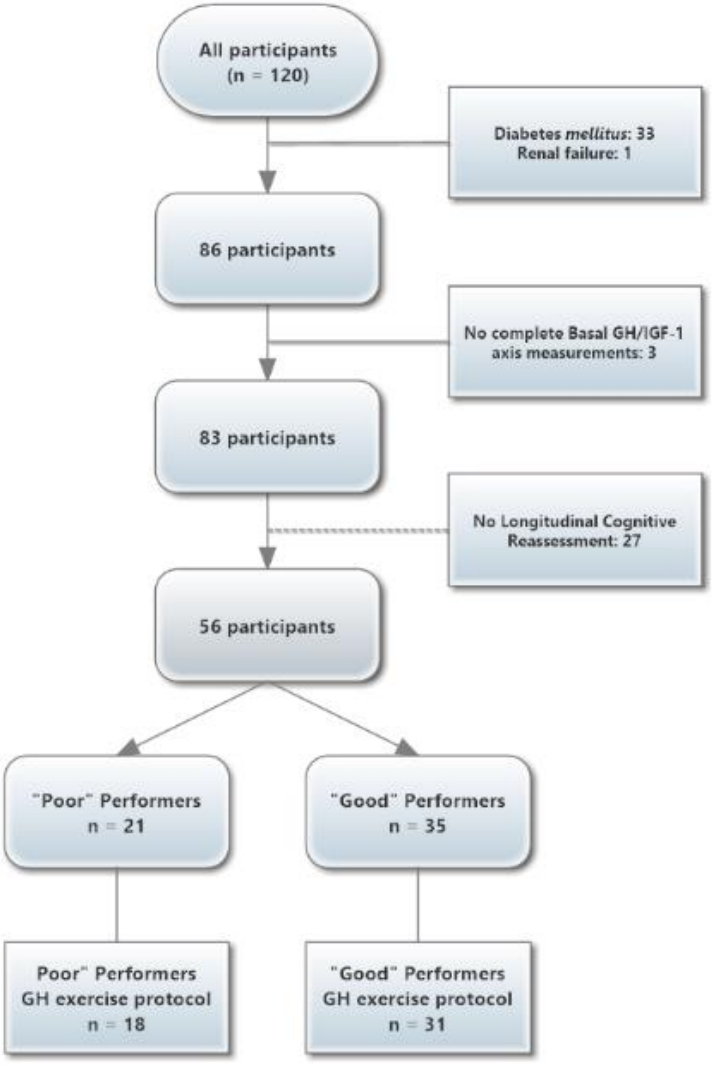

Figure 6.1. Overview of the participant flowchart. GH, growth hormone; IGF-1, insulin-growth factor 1.

Anthropometric measurements

Body weight and height were measured using a standardized method of anthropometric techniques. Body weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm (Tanita® BF350 Body Composition Analyzer, Tanita Corporation, Tokyo, Japan; stadiometer Seca® 217, Seca GmBH & Co Kg, Hamburg, Germany). Body mass index (BMI) was calculated as weight (Kg) / height $(m)^2$. Other anthropometric measures included body-fat mass percentage

measured by bioelectrical impedance analysis (Tanita® BF350 Body Composition Analyzer, Tanita Corporation, Tokyo, Japan).

Analytical methods

All subjects underwent fasting for morning blood collection. The sub-group of participants who were enrolled in the exercise GH stimulus test (see below) were subject to further blood collections, one prior to the test (minimum 4-6 hours after a meal) and another just after finishing the exercise protocol. Plasma concentrations of GH and IGF-1 levels were measured by Immulite® 2000 chemiluminescent immunometric assay. Blood samples were collected, centrifuged and stored at -20°C until hormone levels determination.

Exercise protocol

Acute aerobic exercise is a potent stimulus to GH release (Chang, Dodds et al. 1986). Intensity and duration of exercise, fitness, gender and age influence GH response to exercise (Giustina and Veldhuis 1998). In healthy subjects, a simple regular walking exercise simulation test should, by stimulating GH, help to evaluate the youthful modulation of the GH/IGF-1 axis. With an appropriate exercise, basal serum GH levels are expected to increase above the 7 ng/ml threshold and up to 20-30 ng/ml for approximately 100 -150 min in young adults, with an expected weaker response in older individuals (Pyka, Wiswell et al. 1992, Kanaley, Weltman et al. 2001). During our protocol, an exercise GH-stimulus test was undertaken 4 to 6 h after a meal. The test consisted of 15 minutes of progressive-load exercise (walking in a treadmill) with a Modified Bruce Graded Multistage Protocol. Exclusion criteria were acute illness; non-controlled hypertension; acute coronary ischaemic event less than 6 months; known cardiac, respiratory, renal or hepatic insufficiency; or any physical disability that prevented walking in a treadmill. Heart rate, blood pressure, electrocardiography, pulse oximetry and symptoms were monitored throughout stress testing and recorded at intervals. The test ended after 15 minutes of continuous effort, at maximum exercise tolerated or if participants developed symptoms such as severe disabling chest pain, fatigue, shortness of breath, dizziness, muscle cramps, or any significant excessive rise or fall in blood pressure, impressive ST segment elevation/depression on the electrocardiogram, or induced arrhythmia.

Cognitive assessment

As specified in chapter 3.

Statistical analysis

All data are presented as the mean (median), SD (inter-quartile-range, IQR) for normally (non-normally) distributed data. All continuous variables were checked for normality using the Shapiro-Wilk normality test. Unpaired *t*-test and Mann-Whitney U-test were used to compare continuous variables between the two groups, as appropriate. Wilcoxon matched pairs signed rank test was performed to compare paired variables that failed normality tests. Fisher's exact test was used for categorical variables. Univariate analysis with Pearson correlation coefficient or Spearman's rank correlation was performed, as appropriate, to assess linear relationship between different GH/IGF-1 axis status measurements and the cognitive domain scores (MEM and EXEC) and its temporal trends, stratified by cognitive group. A multiple linear regression analysis was conducted to explore the prediction of Memory/Executive function scores and trends by any GH/IGF-1 axis metrics found relevant in univariate assessment but adjusted to age, gender, baseline cognitive group and education level. Effect size estimates were calculated with Cohen's d and η^2 for continuous variables with parametric and non-parametric comparisons, respectively; *r* for Wilcoxon matched paired test; ϕ coefficient for Fisher's exact test; and R^2 and adjusted R^2 for regression analysis.

All analyses were tested at the 0.05 level of significance and performed using IBM SPSS Statistics, v.21 (IBM, New York USA) and GraphPad Prism, v.6.00 (GraphPad Software, La Jolla California USA). Effect size estimates were evaluated by Cohen's published benchmark classes (small, medium and large) with the following proposed cut-points: 0.2, 0.5 and 0.8 for Cohen's *d*; 0.01, 0.06, 0.14 for η^2; 0.1; 0.3 and 0.5 for *r* and ϕ coefficient; and 0.02, 0.13, 0.26 for R^2 (Cohen 1988).

Results

The final sample included 56 individuals (with 49 fully completing the GH exercise test protocol) with a mean age of 65.5-yr (SD 8.4-yr) and women ratio of 0.46 (n=26). Total study population characteristics are presented in Table 6.1.

Table 6.1. Study population characteristics by cognitive performance group.

Variable	Total (n = 56)	"Poor" (n = 21)	"Good" (n = 35)	*p*	Effect-size
Socio-demographic features					
Sex					
Female, n (%)	26 (46.4)	11 (52.4)	15 (42.9)	0.58	0.09
Age (years), mean (SD)	65.5 (8.4)	67.7 (7.0)	63.7 (8.8)	0.09	0.49
Education level above 4-yr, n (%)	16 (28.6)	1 (4.8)	15 (42.9)	0.002	0.41
Anthropometric features					
Body mass index (Kg/m^2), mean (SD)	29.0 (3.6)	29.9 (3.6)	28.4 (3.6)	0.13	0.42
Body fat mass (%), mean (SD)	32.1 (7.2)	33.3 (7.5)	31.3 (7.0)	0.32	0.28
GH/IGF-1 parameters					
Fasting GH (ng/ml), median (IQR)					
Baseline	0.33 (0.11 – 1.24)	0.32 (0.13 – 1.63)	0.33 (0.11 – 0.87)	0.39	0.01
Last	0.36 (0.11 – 1.32)	0.40 (0.11 – 1.54)	0.27 (0.10 – 1.33)	0.95	<0.01
IGF-1 (ng/ml), mean (SD)					
Baseline	137.2 (45.0)	128.0 (49.0)	142.7 (42.4)	0.24	0.33
Last	124.1 (41.2)	102.0 (38.3)	137.3 (37.4)	0.001	0.93
IGF-1 trend (ng/ml), mean (SD)	-13.1 (42.2)	-26.0 (37.8)	-5.4 (43.3)	0.08	0.50
GH-Exercise Test (ng/ml), median (IQR)*					
Pre-test	0.43 (0.09 – 1.39)	0.31 (0.09 – 1.18)	0.62 (0.16 – 1.82)	0.19	<0.01

Post-test	0.47 (0.17 – 1.99)	0.52 (0.23 – 2.92)	0.37 (0.14 – 1.73)	0.34	0.02
GH-Exercise Test, individuals with increment response, n (%)*	23 (46.9)	9 (50.0)	14 (45.2)	0.77	0.05
Cognitive scores and trends, Median (IQR)					
MEM score					
Baseline	0.26 (-0.91 to 0.81)	-0.98 (-1.34 to -0.58)	0.60 (0.35 to 1.05)	<0.001	0.64
Last	-0.31 (-1.19 to 0.80)	-1.03 (-1.48 to -0.54)	0.32 (-0.74 to 1.17)	<0.001	0.24
EXEC score					
Baseline	0.05 (-0.81 to 0.75)	-0.99 (-1.23 to -0.73)	0.61 (0.35 to 1.05)	<0.001	0.61
Last	0.02 (-0.94 to 0.79)	-1.03 (-1.26 to -0.56)	0.66 (0.20 to 1.31)	<0.001	0.62
MEM score Trend	-0.24 (-0.73 to 0.32)	0.07 (-0.49 to 0.36)	-0.31 (-1.10 to 0.33)	0.05	0.07
EXEC score Trend	0.01 (-0.09 to 0.15)	0.03 (-0.09 to 0.13)	-0.01 (-0.09 to 0.15)	0.92	<0.01

EXEC, executive function dimension composite; GH, growth hormone; IGF-1, Insulin-like growth factor 1; IQR, interquartile range; MEM, memory dimension composite; SD, standard deviation.

* n=49 ("Poor", n=18; "Good", n=31).

On univariate analysis, "Good" performers were tendentially younger (p=0.09, *Cohen's d*=0.49) and had higher education level (p=0.002, ϕ=0.41) than "Poor" performers. No differences were found between the two groups regarding female/male ratio, body mass index or body fat mass percentage. Fasting GH, at baseline and last-visit evaluation, as well as pre- and post-exercise GH levels were also similar between the two groups. Baseline serum concentrations of IGF-1 were also comparable between "Poor" and "Good" performers, but at last-visit evaluation individuals within the "Poor" performers group presented lower levels of IGF-1 (102.0 *vs.* 137.3 ng/ml, p=0.001) with a tendency for steeper decrease in IGF-1 trend overtime (-26.0 *vs.* -5.4 ng/ml, p=0.08) (Figure 6.2).

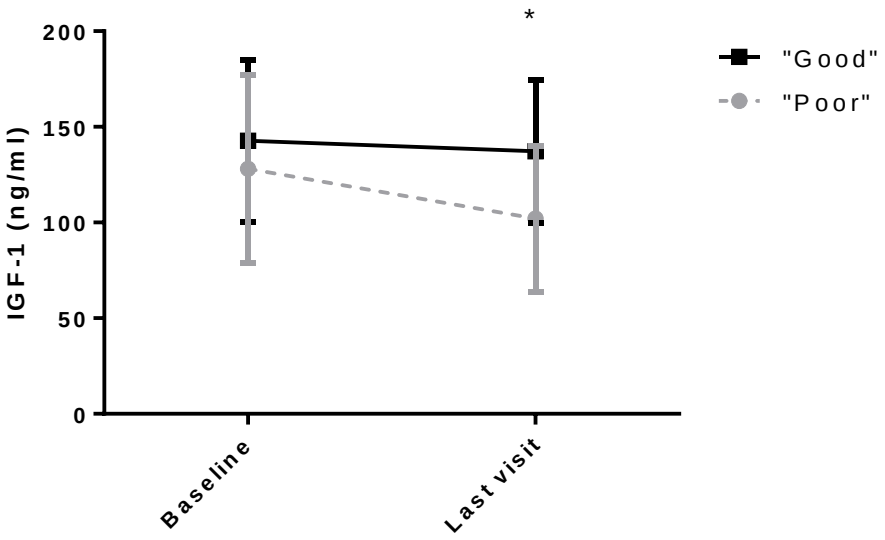

Figure 6.2. IGF-1 levels evolution (mean, SD) within "Good" and "Poor" performers group. IGF-1, Insulin-like growth factor 1. * p=0.001 (last visit "Good" *vs.* "Poor").

The acute aerobic exercise GH-stimulus protocol resulted in GH increment only in 9/18 (50%) and 14/31 (45%) of "Poor" and "Good" performers, respectively, with 4% of the individuals (2/49) reaching the 7 ng/ml threshold (all within the "Poor" group).

Regarding GH/IGF-1 status "stability" between different collections time-points, fasting GH concentrations correlated rather well overtime (r=0.56, p<0.001), just like IGF-1 levels (r=0.52, p<0.001). In the "Poor" group, overall GH/IGF-1 profile consistency with maintenance within the same tercile through both evaluations was 33% (7/21) and 57% (12/21) for fasting GH and IGF-1, respectively. In the "Good" group, overall profile consistency was 54% (19/35) and 49% (17/35) for fasting GH and IGF-1 levels, respectively.

Correlation coefficients weighing cognitive scores and trends with GH/IGF-1 axis hormone measurements are presented in Tables 6.2, 6.3, 6.4 and 6.5.

Table 6.2. Spearman's rank correlation (r_s) between **fasting GH levels** and MEM and EXEC scores and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 21)		**"Good" (n = 35)**	
	Baseline GH	Last GH	Baseline GH	Last GH
Baseline MEM score	0.29	–	-0.17	–
Baseline EXEC score	0.23	–	-0.29	–
Last MEM score	-0.11	-0.11	-0.16	**-0.35***
Last EXEC score	-0.07	0.05	-0.29	**-0.51#**
MEM score trend	-0.33	0.001	-0.19	-0.32
EXEC score trend	-0.38	0.19	-0.15	-0.10

* p=0.04; # p=0.002.

Table 6.3. Pearson's correlation coefficient (r) between **IGF-1** levels and MEM and EXEC scores and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 21)		**"Good" (n = 35)**	
	Baseline IGF-1	Last IGF-1	Baseline IGF-1	Last IGF-1
Baseline MEM score	-0.02	–	0.29	–
Baseline EXEC score	0.11	–	0.17	–
Last MEM score	-0.40	-0.21	0.13	0.08
Last EXEC score	-0.37	-0.34	0.13	-0.12
MEM score trend	**-0.43***	-0.16	0.29	-0.02
EXEC score trend	**-0.61#**	-0.19	-0.08	**-0.33***

* p=0.05; # p=0.003.

Table 6.4. Spearman's rank correlation (r_s) between **post-exercise GH** and MEM and EXEC scores and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 18)	**"Good" (n = 31)**
	Post-Exercise GH	Post-Exercise GH
Baseline MEM score	0.39	-0.05
Baseline EXEC score	**0.65***	-0.30
Last MEM score	-0.14	-0.23
Last EXEC score	0.44	**-0.39#**
MEM score trend	-0.44	-0.32
EXEC score trend	-0.16	**-0.44§**

* p=0.003; # p=0.03; § p=0.01.

Table 6.5. Pearson's correlation coefficient (*r*) between **IGF-1 trend** and MEM and EXEC scores and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" (n = 21)	**"Good" (n = 35)**
	IGF-1 trend	IGF-1 trend
Baseline MEM score	-0.08	-0.14
Baseline EXEC score	-0.39	-0.20
Last MEM score	0.30	**-0.33**#
Last EXEC score	0.14	-0.23
MEM score trend	0.39	-0.30
EXEC score trend	**0.59***	-0.21

* p=0.005; # p=0.05.

Within "Poor" performers, baseline executive composite scores were moderately strong correlated to post-exercise GH levels (r=0.65, p=0.003). In the same group, memory and executive score trends were negatively correlated to baseline IGF-1 (r= -0.43, p=0.05; r= -0.61, p=0.003, respectively). Equally, executive score trend was strongly associated to IGF-1 evolution overtime (r=0.59, p=0.005). Multivariate analysis was performed in order to control for confounding variables (Table 6.6 to 6.13). After adjustment for age, gender and education level, baseline IGF-1 levels and trends maintained significant interactions with EXEC score trend (Tables 6.7 and 6.9).

Table 6.6. Association between observed **last-visit Memory and Executive Function** scores with Age, Gender, Education Level and **last-visit fasting GH levels** predicted by multiple linear regression analysis within the **"Good" performer group**, (n = 35), (last MEM score, p =0.004, R^2 = 0.40, adjusted R^2 = 0.32; EXEC score trend, p <0.001, R^2 = 0.49, adjusted R^2 = 0.43).

	Last MEM score		**Last EXEC score**	
	β (95% CI)	*p*	β (95% CI)	*p*
Age (years)	-0.07 (-0.11 to -0.03)	0.003	-0.04 (-0.06 to -0.01)	0.007
Gender (Exposure: Male)	-0.41 (-1.18 to 0.37)	0.29	0.14 (-0.32 to 0.60)	0.53
Education Level (years)	0.08 (-0.02 to 0.18)	0.11	0.08 (0.02 to 0.13)	0.01
Last fasting GH levels (ng/ml)	0.06 (-0.13 to 0.25)	0.50	0.07 (-0.04 to 0.18)	0.19

Table 6.7. Association between observed **Memory and Executive Function trends** with Age, Gender, Education Level and **baseline IGF-1** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 21), (MEM score trend, p =0.35, R^2 = 0.23, adjusted R^2 = -0.04; EXEC score trend, p =0.03, R^2 = 0.48, adjusted R^2 = 0.35).

	MEM score trend		**EXEC score trend**	
	β (95% CI)	*p*	β (95% CI)	*p*
Age (years)	0.01 (-0.04 to 0.07)	0.60	<0.001 (-0.02 to 0.02)	>0.99
Gender (Exposure: Male)	0.03 (-0.56 to 0.63)	0.91	-0.20 (-0.44 to 0.04)	0.10
Education Level (years)	-0.04 (-0.29 to 0.22)	0.77	0.02 (-0.09 to 0.12)	0.74
Baseline IGF-1 levels (ng/ml)	-0.006 (-0.01 to <0.001)	0.07	**-0.003 (-0.005 to -0.001)**	**0.02**

Table 6.8. Association between observed **Executive Function trend** with Age, Gender, Education Level and **last-visit IGF-1** levels predicted by multiple linear regression analysis within the **"Good" performer group**, (n = 35), (EXEC score trend, p =0.12, R^2 = 0.21, adjusted R^2 = 0.11).

	EXEC score trend	
	β (95% CI)	*p*
Age (years)	0.002 (-0.006 to -0.01)	0.58
Gender (Exposure: Male)	-0.01 (-0.16 to 0.13)	0.86
Education Level (years)	0.02 (-0.001 to 0.03)	0.07
Last IGF-1 levels (ng/ml)	-0.002 (-0.003 to <0.001)	0.09

Table 6.9. Association between observed **Executive Function trend** with Age, Gender, Education Level and **IGF-1 levels trend** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 21), (EXEC score trend, p =0.02, R^2 = 0.52, adjusted R^2 = 0.40).

	EXEC score trend	
	β (95% CI)	*p*
Age (years)	-0.005 (-0.03 to 0.01)	0.58
Gender (Exposure: Male)	-0.22 (-0.45 to 0.003)	0.05
Education Level (years)	<0.001 (-0.10 to 0.10)	>0.99
IGF-1 levels trend (ng/ml)	**0.004 (0.001 to 0.007)**	**0.01**

Within "Good" performers, last-visit memory score was negatively correlated to last fasting GH evaluation (r_s= -0.35, p=0.04) and IGF-1 trend (r_s= -0.33, p=0.05); last executive score was inversely associated to last fasting GH (r_s= -0.51, p=0.002) and to post-exercise GH levels (r_s= -0.39, p=0.03), and the executive score evolution trend was negatively correlated to post-exercise GH and last-visit IGF-1 determination (r_s= -0.44, p=0.01; r_s= -0.33, p=0.05, respectively). After adjustment for age, gender and education level, IGF-1 trend maintained significant interactions with last MEM score; as well as post-exercise GH levels remained negatively associated to last-visit EXEC and EXEC score trend (Table 6.10, 6.12 and 6.13).

Table 6.10. Association between observed **last-visit Memory Function scores** with Age, Gender, Education Level and **IGF-1 levels trend** predicted by multiple linear regression analysis within the **"Good" performer group**, (n = 35), (last-visit MEM score, p =0.001, R^2 = 0.47, adjusted R^2 = 0.39).

	Last MEM score	
	β (95% CI)	*p*
Age (years)	-0.07 (-0.11 to -0.03)	0.001
Gender (Exposure: Male)	-0.23 (-0.95 to 0.50)	0.53
Education Level (years)	0.07 (-0.02 to 0.16)	0.11
IGF-1 levels trend (ng/ml)	-0.008 (-0.02 to <0.001)	0.05

Table 6.11. Association between observed **baseline Executive Function** scores with Age, Gender, Education Level and **post-exercise test GH levels** predicted by multiple linear regression analysis within the **"Poor" performer group**, (n = 18), (baseline EXEC score, p =0.004, R^2 = 0.67, adjusted R^2 = 0.57).

	Baseline EXEC scores	
	β (95% CI)	*p*
Age (years)	-0.01 (-0.03 to 0.01)	0.36
Gender (Exposure: Male)	0.04 (-0.17 to 0.26)	0.68
Education Level (years)	0.11 (0.007 to 0.22)	0.04
Post-exercise GH levels (ng/ml)	0.02 (-0.03 to 0.07)	0.34

Table 6.12. Association between observed **last-visit Executive Function** scores with Age, Gender, Education Level and **post-exercise test GH levels** predicted by multiple linear regression analysis within the **"Good" performer group**, (n = 31), (last-visit EXEC score, p =0.001, R^2 = 0.51, adjusted R^2 = 0.43).

	Last EXEC scores	
	β (95% CI)	p
Age (years)	-0.03 (-0.05 to -0.005)	0.02
Gender (Exposure: Male)	0.09 (-0.34 to 0.51)	0.68
Education Level (years)	0.06 (0.02 to 0.11)	0.01
Post-exercise GH levels (ng/ml)	**-0.14 (-0.25 to -0.02)**	**0.02**

Table 6.13. Association between observed **Executive Function trend** with Age, Gender, Education Level and **post-exercise test GH levels** predicted by multiple linear regression analysis within the **"Good" performer group**, (n = 31), (EXEC score trend, p =0.01, R^2 = 0.38, adjusted R^2 = 0.28).

	EXEC trend	
	β (95% CI)	p
Age (years)	0.005 (-0.003 to 0.01)	0.23
Gender (Exposure: Male)	-0.04 (-0.18 to 0.10)	0.60
Education Level (years)	0.02 (0.004 to 0.04)	0.02
Post-exercise GH levels (ng/ml)	**-0.05 (-0.09 to -0.02)**	**0.006**

Discussion

In this older population-based study, healthy "Poor" cognitive performers presented lower levels of IGF-1 during the follow-up and baseline IGF-1 levels and temporal trend were independently associated to executive cognitive performance evolution overtime. Interestingly, in the "Good" performers, the downward IGF-1 trend was only independently linked to memory performance

score at the last visit. Moreover, in this "Good" group and after relevant covariate adjustments, post-exercise GH levels were negatively associated to last executive cognitive performance and to its temporal variation.

Cross-sectionally, total IGF-1 has been linked to better global cognitive function, particularly in women (Wennberg, Hagen et al. 2018). Some few longitudinal studies exploring this association presented an inverse-U curve connection. In an 8-year follow-up study with 400 middle-age men, *Tumati et al.* found that both higher and lower levels of IGF-1 at baseline were associated to worse future global cognitive performance (measured by MMSE) and with lower concurrent processing capacity, particularly in older individuals (Tumati, Burger et al. 2016). In the present study, baseline IGF-1 levels in the "Poor" cognitive group were negatively associated to executive performance overtime. This apparent paradox was also reported by some authors and maybe justified, in our case, by the following (Tumati, Burger et al. 2016). Baseline IGF-1 in "Poor" performers already started at lower levels and presented a sharper temporal decline when compared to "Good" performers. This feature may potentiate a "bottom" effect, when individuals near the lower limit stay rather stable above it and only individuals with somewhat higher IGF-1 serum concentrations will reveal IGF-1 decline and evolve to worse outcomes. Nevertheless, a potential consequence of IGF-1 positive influence over cognition was also observed when, within this group, IGF-1 levels evolution overtime was positively associated to their executive function trend. Meaning that, during the follow-up, "Poor" cognitive performers with greater decline in IGF-1 were also at risk of greater fall in their executive composite scores. No such effect was observed in the "Good" performers group. It is possible that one of the reasons for executive tasks susceptibility to IGF-1 within "Poor" cognitive performers may rest on their somewhat "depleted" executive functioning reserve and increased sensitivity to modulation by the GH/IGF-1 axis. To our knowledge, no previous studies have assessed IGF-1 levels during longitudinal cohorts and their association to overall cognitive functioning.

To date, all published memory cognitive outcomes evaluated in healthy adult cohorts presented no association to GH/IGF-1 levels (Frater, Lie et al. 2018). In our study, memory composite score at the last visit was inversely associated to IGF-1 levels only in the "Good" performers group. No clear explanation for this observation is available. GH and IGF-1 receptors are present in the hippocampus during ageing and are considered essential for the acute brain-injury recovery responses but not to chronic neuro-degenerative conditions (Nyberg and Burman 1996, Zhu, Fan et al. 2008, Ashpole, Sanders et al. 2015, Gubbi, Quipildor et al. 2018). Subnormal GH/IGF-1

signaling seems protective in some Alzheimer's disease models (Trueba-Saiz, Cavada et al. 2013). If this local feature is somewhat linked to our observed negative relationship between memory performance and serum IGF-1 levels trend, it is not known.

To our knowledge, GH response to exercise has never been tested in cognitive prospective studies. In our study, only two participants surpassed the expected "young" threshold. This is in line with the known weaker response in older individuals but it may also be that these individuals develop some exercise-resistant response and therefore fail to respond properly to this stimulus (Weltman, Weltman et al. 2006). Only few studies explored GH enhancement and cognitive outcomes in older healthy individuals, and only two trials using GHRH analogue administration found any improvement in executive functioning, particularly working memory (Vitiello, Moe et al. 2006, Baker, Barsness et al. 2012). In our study, higher post-exercise GH levels were associated, only in the "Good" performers, to lower executive performance at the last visit but also to an increased decline in executive functioning trends during follow-up. This apparent negative association may reflect an exercise-GH-resistant protective effect. However, since very few participants did produce a "normal" GH response to exercise, it could be speculated that only those who surpassed the exercise-stress limit had actually been properly stimulated and that all others were potentially more fit and failed to be appropriately tested. This may have resulted in a bias selection of the less-fit responders and therefore help explain such a negative GH-enhancement connection to executive function tasks.

Some other important limitations are present in this study. First, due to the small number of participants included with full longitudinal assessment it is possible that the sensitivity to detect important differences between the two distinct cognitive groups is compromised. Second, there was a relatively short time between the two cognitive evaluations (min-max: 16-22 months), which may have also impaired the ability to detect any intra or inter-individual cognitive time-dependent differences, underestimating potential associations with GH/IGF-1 axis status measurement.

One of the main strengths of this study is the longitudinally two-point GH/IGF-1 axis assessment. Data about this longitudinal hormone axis performance is not usually collected and reported. Reviewing associations between cognitive performances and GH/IGF-1 values, sometimes collected decades before, may in part invalidate many of the published conclusions. Another important feature is the physiological testing of GH response to exercise. Despite the limitations addressed above, this is the first study to evaluate prospectively this type of provocative test with cognitive performance in a healthy older population. Other major strengths are grounded on the

exhaustive cognitive evaluation performed longitudinally with the construction of coherent executive and memory cognitive domain composites assessment.

In conclusion, adjusted baseline higher levels of IGF-1 are associated to worse executive functioning evolution overtime in "Poor" cognitive performers, while individual IGF-1 trends during follow-up are positively related to concurrent executive composite evolution. Worse executive task scores and tendencies during ageing are connected to higher GH-exercise responses in "Good" cognitive performers. A negative interaction was also found between IGF-1 temporal trajectory and last visit memory scores, but only within participants with better preserved cognitive abilities.

This complex and apparent paradoxical connection between GH/IGF-1 status and cognitive functioning in healthy older individuals warrants a better understanding between adaptative age-related adjustments and normal brain ageing process. Future relevant studies are needed to address the role of time-window exposure, intra-individually hormonal axis trajectories and particularly "chronic" *versus* "acute" local brain exposure to GH and/or IGF-1.

7. Discussion and future perspectives

In this thesis, I have examined four hormonal systems previously proposed as contributing to ageing and to cognitive performance, namely the vitamin D (chapter 3), hypothalamus-pituitary-adrenal (chapter 4), hypothalamus-pituitary-thyroid (chapter 5) and the GH/IGF-1 axes (chapter 6).

I will next discuss the key findings and limitations in the context of current scientific literature. Finally, directions for future practice and research will be considered.

Key-findings and relevance

Vitamin D

Key-findings:

1. Median vitamin D levels in the cohort were below the recommended standards for adult population and were not different between better and worse cognitive performers;
2. In this longitudinal study with healthy non-demented older individuals, no association was found between standardized season-adjusted 25(OH)D levels and the variation of the memory and executive cognitive domains.

Relevance:

These findings add up to the most recent literature and the few studies that evaluated cognitive performance in populations exclusively with vitamin D insufficiency like ours. Some authors proposed that higher 25(OH)D thresholds (well above most of the ones found in our participants) are needed to demonstrate its influence over cognitive performance but the very few reports that tried to use lower cut-points have failed to present any clear positive association (Annweiler, Schott et al. 2010, Llewellyn, Lang et al. 2011, Annweiler, Annweiler et al. 2014). Some inconsistencies are also recognised by longer longitudinal studies. In a recent large cohort submitted to extensive neuro-cognitive evaluation, it was found that after a mean 5-year follow-up higher baseline serum 25(OH)D concentrations were associated to a slower rate of decline in verbal fluency, whereas in a recent 20-year follow-up study, mid-life 25(OH)D measurements were not associated with cognitive decline (Beydoun, Hossain et al. 2018, Schneider, Zhao et al. 2018).

Therefore, it seems that differences of 25(OH)D levels, within below "sufficiency" limits, in a healthy older cohort are not associated to distinctive longitudinal cognitive trends.

HPA axis

Key-findings:

1. No differences were found between morning and nocturnal cortisol secretion between the "Good" and "Poor" cognitive performers;
2. Unadjusted higher nocturnal cortisol levels were associated to next-day worse executive performance, but only in the "Good" performers;
3. In the longitudinal analysis, no association was found between morning and nocturnal cortisol secretion with the variation in the memory and executive cognitive domains.

Relevance:

Despite important limitations (see below), these findings are in accordance with the studies that evaluated cognitive performance in older populations. To date most reports have connected nocturnal HPA axis hyperactivation with worse cognitive performance. Despite no previous publication exploring cognitive outcomes with nocturnal urinary cortisol assessment, some large healthy cohort studies did report similar negative cross-sectional associations between nocturnal salivary cortisol levels and poorer executive functioning (Geerlings, Sigurdsson et al. 2015). A much less clear picture is provided by the few longitudinal studies; several, like ours, failing to show a coherent link between morning or evening cortisol levels and cognitive decline (Franz, O'Brien et al. 2011, Singh-Manoux, Dugravot et al. 2014).

In conclusion, despite some data indicating that executive tasks in older individuals with better cognitive performance are more susceptible to the negative influence of higher nocturnal cortisol secretion, the adjusted results states that, in this healthy older cohort, nocturnal and morning cortisol levels (within normal range) are not associated to distinctive longitudinal cognitive trends.

HPT axis

Key-findings:

1. Median urinary iodine data defined this cohort as mildly iodine-deficient;
2. No differences were found between thyroid function and morphology between "Good" and "Poor" performers;
3. Unadjusted TSH levels were positively associated to parallel executive cognitive functioning, but only in the "Poor" performers;
4. Unadjusted free T_4 levels were negatively associated to executive and memory tasks, but only in the "Poor" performers;
5. In the longitudinal study, no association was found between thyroid function and changes in memory and executive cognitive domains.
6. All associations were lost upon adjustment for age, gender and education.

Relevance:

In accordance to these results, the Minho region (where the study population resides) and the rest of Portugal has been previously defined as mild iodine-deficient (Costeira, Oliveira et al. 2010, Limbert, Prazeres et al. 2012).

Despite neutral findings in the adjusted models, our unadjusted results are in line with the studies that evaluated cognitive performance in older populations. Several cross-sectional studies described a positive association between TSH and memory or executive functions, even after multiple adjustments (Wahlin, Wahlin et al. 1998, Beydoun, Beydoun et al. 2012). Even with some contradictory results in younger individuals, large euthyroid older prospective cohorts have also shown that higher free T_4 levels were associated to worse global cognitive scores and an increased risk of dementia (Hogervorst, Huppert et al. 2008, Yeap, Alfonso et al. 2012).

In summary, despite the loss of any association upon relevant adjustments, these results seems to add up to the idea that, in older healthy cohorts (even if iodine deficient), TSH levels (within normal range) are positively associated to executive functioning and that FT_4 is inversely associated to both executive and memory tasks.

GH/IGF-1 axis

Key-findings:

1. Over time better cognitive preserved individuals present higher IGF-1 levels;
2. In adjusted models, low baseline levels of IGF-1 were associated to better executive functioning in the "Poor" performers;
3. Steeper IGF-1 age-related decline seems to be longitudinally associated with worse executive cognitive trends;
4. In adjusted models, post-exercise GH levels were negatively related to executive performance over time in the "Good" performers.

Relevance:

Populational studies have shown that normal ageing is related to an overall decrease in GH secretion and to a steady decrease in IGF-1 levels, with some noticeable inter-individual variation (Veldhuis 2013). Our results are in line with this observation and recognized also that better cognitive-preserved participants present higher ("younger"?) IGF-1 levels.

Regardless of this finding, within each group higher IGF-1 levels were not associated to better cognitive performance. Actually, in the "Poor" performers group low baseline levels of IGF-1 were associated to superior executive functioning. Contrary to our findings, and despite some gender effect, previous cross-sectional studies have found that higher IGF-1 levels were associated to higher global cognitive function (Wennberg, Hagen et al. 2018). Large longitudinal studies have found that both higher and lower levels of IGF-1 at baseline were associated to worse future global cognitive performance with an inverse-U curve connection (Tumati, Burger et al. 2016). Our paradox finding within the "Poor" cognitive performers has no obvious explanation but it seems to be partially in line with this observation whereas individuals with low IGF-1 levels may perform cognitively better than those with higher IGF-1, despite both being outperformed by intermediate levels.

To our knowledge, no previous studies have explored IGF-1 levels performance during longitudinal cohorts and its link to overall cognitive functioning, so the finding that steeper IGF-1 decline is connected to worse cognitive outcomes is novel. Also original is the post-exercise GH assessment connecting somatotropic axis and cognitive outcomes. GH stimulation by GHRH analogue administration was described to improve executive functioning (Vitiello, Moe et al. 2006, Baker, Barsness et al. 2012). Our conflicting result is not easily explained. It could be speculated that

most of the individuals tested did not surpass the exercise-stress limit and that they may have biased the sample by placing the most fit individuals within the non-responder's group. It is recognized that individuals that do not achieve exercise tolerance thresholds may not be fully "stimulated" by the exercise protocol and therefore may lack the expected surge in GH secretion. Consequently, if the final analysis was not adjusted to their exercise fatigue scores it is possible that some unbalance distribution of better fit individuals could occur and influenced the results.

In conclusion, IGF-1 levels are higher in individuals with better preserved cognitive function. Despite this general statement, some of our data seems to indicate that longitudinal executive task decline in older individuals with worse cognitive performance is negatively associated to higher baseline IGF-1 levels. Additionally, higher stimulated GH levels, this time in the "Good" performers group, are independently associated to worse executive scores and its time-dependent decline.

Limitations

Some important limitations were recognized in this work. There was an overall reduced number of participants available for final exploration with a complete longitudinal cognitive assessment, which decreased the sensitivity to detect any small effect and prevented further analysis when comparing distinct groups based on more extreme hormonal distribution. This was particularly relevant when a more homogeneous distribution of values was observed between the two groups, such as in 25(OH)D, adrenal and thyroid measurements.

There was also a relatively short time between the two longitudinal cognitive evaluations (mean time 18 months), which may have impaired even further the ability to detect more subtle intra or inter-individual cognitive time-dependent differences, therefore underestimating potential associations with the endocrine features. Also given the relatively low number of sample collections (between one and two) it is plausible that they were insufficient to reveal individual endocrine axis set-points and may have led to understate intra-individual variation and hindered some of the conclusions.

Other limitations were rather specific. Exploring vitamin D, some problems were initially found with the routine 25(OH)D determination protocol. The need for better analytical performance was obtained during the study by incorporating high-performance liquid chromatography-tandem mass spectrometry methodology, which provided a more accurate measure of vitamin D status when comparing to the immunological techniques commonly used. Also, despite the two seasonal

opposite 25(OH)D determinations, no prolonged longitudinal evaluation of its levels was made. This may have resulted in a rather "late-life" estimation of vitamin D status and limited the accuracy of establishing any longitudinal effect on cognition. Another limitation was that our population was almost homogenously vitamin D insufficient which made it harder to uncover any associations, given the lack of 25(OH)D levels inter-individual variability found within the cohort.

Investigating the HPT axis, we have defined this population as iodine deficient. This makes it harder to compare with the literature, since almost all published studies were done in iodine-sufficient settings and no known report has ever explored cognitive performances in older population with such degree of iodine deficiency.

With the expansion of population ageing, much comprehensive knowledge must be produced in order to better understand healthy senescence. These studies contribute to existing insight of the connections between the endocrine system and cognitive functioning. For example, from the present work it seems that late-life hormone-cognitive modulation may be different in extremes of cognitive performance within the healthy spectrum, in subtle and sometimes paradoxically ways. The search for the underlying mechanisms and actual role of each hormone axis will pave future studies.

Conclusion remarks and future directions

Ageing is a complex occurrence with recognized impact on several hormone axes and cognitive functioning. Genetic traits and life-events epigenetic influences have the ability of determine individual ageing and cognitive trail. Much like paediatric growth charts, each person may present a unique biography of underlying homeostatic mechanisms within "normative" percentiles (figure 7.1). As with many other systems, late-life endocrine axes signatures, some of which were here explored, may just represent the needed physiological adjustments to get to mid-age. These will fill some pages of each individual life-book, probably determining some of the obvious differences between elderly people, but not so much on a causal perspective.

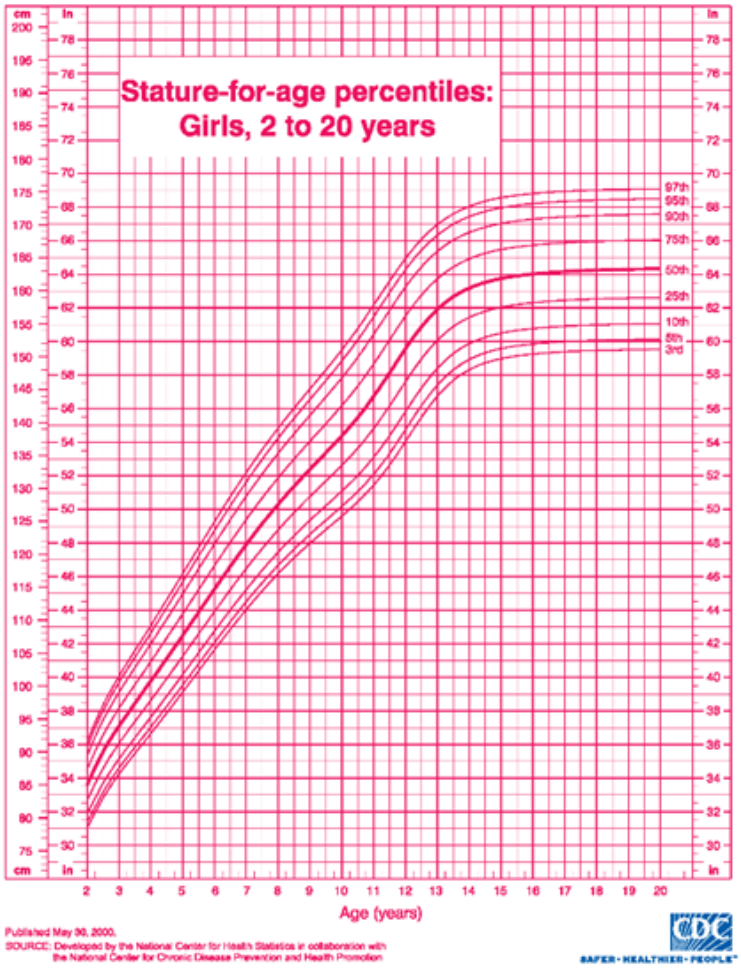

Figure 7.1. Centre for Disease Control stature-for-age percentiles chart for girls. Source: 2000 CDC growth charts for the United States of America: methods and development.

Further studies are required whereby issues like earlier cognitive and endocrine performances with longer follow-up cohorts. Similarly, considerations on the biological variation should be viewed with attention to non-linear cognitive and endocrine dynamics.

Such endeavour is hard and methodologically difficult but with some adaptations by using surrogate markers of *longitudinal* endocrine and cognitive performance (*e.g.* hair cortisol levels collected during lifetime, blood banks, school and pre-employment cognitive test results) it could become more feasible. Only then will it be possible to recognize more clearly the link between all these endocrine axes and ageing, allowing for the development of strategies for true healthy ageing.

Figure 7.2. The Sandai-Shogun-no-matsu in the Tokyo Palace collection in Japan is a white pine Bonsai believed to be over 500 years old. With skill and discipline any species of tree can be grown to become a Bonsai. Similarly, in humans, special care is required to achieve the desired goal of optimal lifespan with healthy ageing.

Ya somos el olvido que seremos.
El polvo elemental que nos ignora
y que fue el rojo Adán y que es ahora
todos los hombres y los que seremos.

Ya somos en la tumba las dos fechas
del principio y el término. La caja,
la obscena corrupción y la mortaja,
los triunfos de la muerte y las endechas.

No soy el insensato que se aferra
al mágico sonido de su nombre;
pienso con esperanza en aquel hombre
que no sabrá quien fui sobre la tierra.

Bajo el indiferente azul del cielo,
esta meditación es un consuelo.

Aquí. Hoy.
Jorge Luís Borges, 1899-1986.

8. References

(2018). "Britannica, Encyclopedia." Retrieved 05.2018, from https://www.britannica.com/.

Abbott, D., D. Rotnem, M. Genel and D. J. Cohen (1982). "Cognitive and emotional functioning in hypopituitary short-statured children." Schizophr Bull **8**(2): 310-319.

Abuzzahab, M. J., A. Schneider, A. Goddard, F. Grigorescu, C. Lautier, E. Keller, W. Kiess, J. Klammt, J. Kratzsch, D. Osgood, R. Pfaffle, K. Raile, B. Seidel, R. J. Smith, S. D. Chernausek and G. Intrauterine Growth Retardation Study (2003). "IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation." N Engl J Med **349**(23): 2211-2222.

Adams, J. S. and M. Hewison (2012). "Extrarenal expression of the 25-hydroxyvitamin D-1-hydroxylase." Arch Biochem Biophys **523**(1): 95-102.

Aguiar-Oliveira, M. H. and A. Bartke (2019). "Growth Hormone Deficiency: Health and Longevity." Endocr Rev **40**(2): 575-601.

Aguiar-Oliveira, M. H., F. T. Oliveira, R. M. Pereira, C. R. Oliveira, A. Blackford, E. H. Valenca, E. G. Santos, M. B. Gois-Junior, R. A. Meneguz-Moreno, V. P. Araujo, L. A. Oliveira-Neto, R. P. Almeida, M. A. Santos, N. T. Farias, D. C. Silveira, G. W. Cabral, F. R. Calazans, J. D. Seabra, T. F. Lopes, E. O. Rodrigues, L. A. Porto, I. P. Oliveira, E. V. Melo, M. Martari and R. Salvatori (2010). "Longevity in untreated congenital growth hormone deficiency due to a homozygous mutation in the GHRH receptor gene." J Clin Endocrinol Metab **95**(2): 714-721.

Ahern, T. and F. C. Wu (2015). "New horizons in testosterone and the ageing male." Age Ageing **44**(2): 188-195.

Akbaraly, T. N., I. Hininger-Favier, I. Carriere, J. Arnaud, V. Gourlet, A. M. Roussel and C. Berr (2007). "Plasma selenium over time and cognitive decline in the elderly." Epidemiology **18**(1): 52-58.

Akhtar, S. (2017). Diseases of aging. Stoelting's Anesthesia and Co-Existing Disease. Roberta Hines and K. Marschall, Elsevier.

Akintola, A. A., S. W. Jansen, D. van Bodegom, J. van der Grond, R. G. Westendorp, A. J. de Craen and D. van Heemst (2015). "Subclinical hypothyroidism and cognitive function in people over 60 years: a systematic review and meta-analysis." Front Aging Neurosci **7**: 150.

Al-Delaimy, W. K., D. von Muhlen and E. Barrett-Connor (2009). "Insulinlike growth factor-1, insulinlike growth factor binding protein-1, and cognitive function in older men and women." J Am Geriatr Soc **57**(8): 1441-1446.

Albert, M. S., K. Jones, C. R. Savage, L. Berkman, T. Seeman, D. Blazer and J. W. Rowe (1995). "Predictors of cognitive change in older persons: MacArthur studies of successful aging." Psychol Aging **10**(4): 578-589.

Almela, M., L. van der Meij, V. Hidalgo, C. Villada and A. Salvador (2012). "The cortisol awakening response and memory performance in older men and women." Psychoneuroendocrinology **37**(12): 1929-1940.

Aloia, J. F. (2011). "Clinical Review: The 2011 report on dietary reference intake for vitamin D: where do we go from here?" J Clin Endocrinol Metab **96**(10): 2987-2996.

Alvarez, J. A., R. Chowdhury, D. P. Jones, G. S. Martin, K. L. Brigham, J. N. Binongo, T. R. Ziegler and V. Tangpricha (2014). "Vitamin D status is independently associated with plasma glutathione and cysteine thiol/disulphide redox status in adults." Clin Endocrinol (Oxf) **81**(3): 458-466.

Amaral, D. G. (1993). "Morphological analyses of the brains of behaviorally characterized aged nonhuman primates." Neurobiol Aging **14**(6): 671-672.

Amso, D. and G. Scerif (2015). "The attentive brain: insights from developmental cognitive neuroscience." Nat Rev Neurosci **16**(10): 606-619.

Anastasiou, C. A., M. Yannakoulia and N. Scarmeas (2014). "Vitamin D and cognition: an update of the current evidence." J Alzheimers Dis **42 Suppl 3**: S71-80.
Andersen-Ranberg, K., B. Jeune, M. Hoier-Madsen and L. Hegedus (1999). "Thyroid function, morphology and prevalence of thyroid disease in a population-based study of Danish centenarians." J Am Geriatr Soc **47**(10): 1238-1243.
Andersen, S., K. M. Pedersen, N. H. Bruun and P. Laurberg (2002). "Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease." J Clin Endocrinol Metab **87**(3): 1068-1072.
Andersen, S. L., P. Sebastiani, D. A. Dworkis, L. Feldman and T. T. Perls (2012). "Health span approximates life span among many supercentenarians: compression of morbidity at the approximate limit of life span." J Gerontol A Biol Sci Med Sci **67**(4): 395-405.
Andrew, R., D. I. Phillips and B. R. Walker (1998). "Obesity and gender influence cortisol secretion and metabolism in man." J Clin Endocrinol Metab **83**(5): 1806-1809.
Angelini, A., C. Bendini, F. Neviani, L. Bergamini, B. Manni, T. Trenti, R. Rovati and M. Neri (2009). "Insulin-like growth factor-1 (IGF-1): relation with cognitive functioning and neuroimaging marker of brain damage in a sample of hypertensive elderly subjects." Arch Gerontol Geriatr **49 Suppl 1**: 5-12.
Annweiler, C., T. Annweiler, M. Montero-Odasso, R. Bartha and O. Beauchet (2014). "Vitamin D and brain volumetric changes: Systematic review and meta-analysis." Maturitas **78**(1): 30-39.
Annweiler, C., R. Bartha, S. Goncalves, S. N. Karras, P. Millet, F. Feron and O. Beauchet (2015). "Vitamin D-related changes in intracranial volume in older adults: a quantitative neuroimaging study." Maturitas **80**(3): 312-317.
Annweiler, C. and O. Beauchet (2014). "Vitamin d in older adults: the need to specify standard values with respect to cognition." Front Aging Neurosci **6**: 72.
Annweiler, C., D. Milea, H. E. Whitson, C. Y. Cheng, T. Y. Wong, M. K. Ikram, E. L. Lamoureux and C. Sabanayagam (2016). "Vitamin D insufficiency and cognitive impairment in Asians: a multi-ethnic population-based study and meta-analysis." J Intern Med **280**(3): 300-311.
Annweiler, C., M. Montero-Odasso, D. J. Llewellyn, S. Richard-Devantoy, G. Duque and O. Beauchet (2013). "Meta-analysis of memory and executive dysfunctions in relation to vitamin D." J Alzheimers Dis **37**(1): 147-171.
Annweiler, C., A. M. Schott, G. Allali, S. A. Bridenbaugh, R. W. Kressig, P. Allain, F. R. Herrmann and O. Beauchet (2010). "Association of vitamin D deficiency with cognitive impairment in older women: cross-sectional study." Neurology **74**(1): 27-32.
Annweiler, C., A. M. Schott, Y. Rolland, H. Blain, F. R. Herrmann and O. Beauchet (2010). "Dietary intake of vitamin D and cognition in older women: a large population-based study." Neurology **75**(20): 1810-1816.
Annweiler, C., J. C. Souberbielle, A. M. Schott, L. de Decker, G. Berrut and O. Beauchet (2011). "[Vitamin D in the elderly: 5 points to remember]." Geriatr Psychol Neuropsychiatr Vieil **9**(3): 259-267.
Anyetei-Anum, C. S., V. R. Roggero and L. A. Allison (2018). "Thyroid hormone receptor localization in target tissues." J Endocrinol **237**(1): R19-R34.
Araujo, A. B. and G. A. Wittert (2011). "Endocrinology of the aging male." Best Pract Res Clin Endocrinol Metab **25**(2): 303-319.
Ardila, A. (2007). "Normal aging increases cognitive heterogeneity: analysis of dispersion in WAIS-III scores across age." Arch Clin Neuropsychol **22**(8): 1003-1011.
Arnsten, A. F., J. X. Cai and P. S. Goldman-Rakic (1988). "The alpha-2 adrenergic agonist guanfacine improves memory in aged monkeys without sedative or hypotensive side effects: evidence for alpha-2 receptor subtypes." J Neurosci **8**(11): 4287-4298.

Arwert, L. I., J. B. Deijen and M. L. Drent (2003). "Effects of an oral mixture containing glycine, glutamine and niacin on memory, GH and IGF-I secretion in middle-aged and elderly subjects." Nutr Neurosci **6**(5): 269-275.
Arwert, L. I., J. B. Deijen, J. Witlox and M. L. Drent (2005). "The influence of growth hormone (GH) substitution on patient-reported outcomes and cognitive functions in GH-deficient patients: a meta-analysis." Growth Horm IGF Res **15**(1): 47-54.
Arwert, L. I., D. J. Veltman, J. B. Deijen, P. S. van Dam, H. A. Delemarre-van deWaal and M. L. Drent (2005). "Growth hormone deficiency and memory functioning in adults visualized by functional magnetic resonance imaging." Neuroendocrinology **82**(1): 32-40.
Arwert, L. I., D. J. Veltman, J. B. Deijen, P. S. van Dam and M. L. Drent (2006). "Effects of growth hormone substitution therapy on cognitive functioning in growth hormone deficient patients: a functional MRI study." Neuroendocrinology **83**(1): 12-19.
Ashpole, N. M., S. Logan, A. Yabluchanskiy, M. C. Mitschelen, H. Yan, J. A. Farley, E. L. Hodges, Z. Ungvari, A. Csiszar, S. Chen, C. Georgescu, G. B. Hubbard, Y. Ikeno and W. E. Sonntag (2017). "IGF-1 has sexually dimorphic, pleiotropic, and time-dependent effects on healthspan, pathology, and lifespan." Geroscience **39**(2): 129-145.
Ashpole, N. M., J. E. Sanders, E. L. Hodges, H. Yan and W. E. Sonntag (2015). "Growth hormone, insulin-like growth factor-1 and the aging brain." Exp Gerontol **68**: 76-81.
Assmann, K. E., M. Touvier, V. A. Andreeva, M. Deschasaux, T. Constans, S. Hercberg, P. Galan and E. Kesse-Guyot (2015). "Midlife plasma vitamin D concentrations and performance in different cognitive domains assessed 13 years later." Br J Nutr **113**(10): 1628-1637.
Audenaert, K., B. Brans, K. Van Laere, P. Lahorte, J. Versijpt, K. van Heeringen and R. Dierckx (2000). "Verbal fluency as a prefrontal activation probe: a validation study using 99mTc-ECD brain SPET." Eur J Nucl Med **27**(12): 1800-1808.
Aung, K., J. Burnett, S. M. Smith and C. B. Dyer (2006). "Vitamin D deficiency associated with self-neglect in the elderly." J Elder Abuse Negl **18**(4): 63-78.
Auso, E., R. Lavado-Autric, E. Cuevas, F. E. Del Rey, G. Morreale De Escobar and P. Berbel (2004). "A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration." Endocrinology **145**(9): 4037-4047.
Ayuk, J. and M. C. Sheppard (2008). "Does acromegaly enhance mortality?" Rev Endocr Metab Disord **9**(1): 33-39.
Babiloni, C., A. Ferretti, C. Del Gratta, F. Carducci, F. Vecchio, G. L. Romani and P. M. Rossini (2005). "Human cortical responses during one-bit delayed-response tasks: an fMRI study." Brain Res Bull **65**(5): 383-390.
Babson, K. A., S. H. Woodward, M. Schaer, S. E. Sephton and D. G. Kaloupek (2017). "Salivary Cortisol and Regional Brain Volumes Among Veterans With and Without Posttraumatic Stress Disorder." Biol Psychiatry Cogn Neurosci Neuroimaging **2**(4): 372-379.
Bach, L. A. (2018). "IGF-binding proteins." J Mol Endocrinol **61**(1): T11-T28.
Bae, H., A. Gurinovich, A. Malovini, G. Atzmon, S. L. Andersen, F. Villa, N. Barzilai, A. Puca, T. T. Perls and P. Sebastiani (2018). "Effects of FOXO3 Polymorphisms on Survival to Extreme Longevity in Four Centenarian Studies." J Gerontol A Biol Sci Med Sci **73**(11): 1439-1447.
Baker, D. J., M. M. Dawlaty, T. Wijshake, K. B. Jeganathan, L. Malureanu, J. H. van Ree, R. Crespo-Diaz, S. Reyes, L. Seaburg, V. Shapiro, A. Behfar, A. Terzic, B. van de Sluis and J. M. van Deursen (2013). "Increased expression of BubR1 protects against aneuploidy and cancer and extends healthy lifespan." Nat Cell Biol **15**(1): 96-102.
Baker, L. D., S. M. Barsness, S. Borson, G. R. Merriam, S. D. Friedman, S. Craft and M. V. Vitiello (2012). "Effects of growth hormone-releasing hormone on cognitive function in adults with mild

cognitive impairment and healthy older adults: results of a controlled trial." Arch Neurol **69**(11): 1420-1429.
Baker, M. R., M. Peacock and B. E. Nordin (1980). "The decline in vitamin D status with age." Age Ageing **9**(4): 249-252.
Baldini, I. M., A. Vita, M. C. Mauri, V. Amodei, M. Carrisi, S. Bravin and L. Cantalamessa (1997). "Psychopathological and cognitive features in subclinical hypothyroidism." Prog Neuropsychopharmacol Biol Psychiatry **21**(6): 925-935.
Baldo, J. V., A. P. Shimamura, D. C. Delis, J. Kramer and E. Kaplan (2001). "Verbal and design fluency in patients with frontal lobe lesions." J Int Neuropsychol Soc **7**(5): 586-596.
Balion, C., L. E. Griffith, L. Strifler, M. Henderson, C. Patterson, G. Heckman, D. J. Llewellyn and P. Raina (2012). "Vitamin D, cognition, and dementia: a systematic review and meta-analysis." Neurology **79**(13): 1397-1405.
Ballotti, R., F. C. Nielsen, N. Pringle, A. Kowalski, W. D. Richardson, E. Van Obberghen and S. Gammeltoft (1987). "Insulin-like growth factor I in cultured rat astrocytes: expression of the gene, and receptor tyrosine kinase." EMBO J **6**(12): 3633-3639.
Barbi, E., F. Lagona, M. Marsili, J. W. Vaupel and K. W. Wachter (2018). "The plateau of human mortality: Demography of longevity pioneers." Science **360**(6396): 1459-1461.
Barnes, P. J. (2010). "Mechanisms and resistance in glucocorticoid control of inflammation." J Steroid Biochem Mol Biol **120**(2-3): 76-85.
Barrere, X., P. Valeix, P. Preziosi, M. Bensimon, B. Pelletier, P. Galan and S. Hercberg (2000). "Determinants of thyroid volume in healthy French adults participating in the SU.VI.MAX cohort." Clin Endocrinol (Oxf) **52**(3): 273-278.
Barron, A. M. and C. J. Pike (2012). "Sex hormones, aging, and Alzheimer's disease." Front Biosci (Elite Ed) **4**: 976-997.
Barroso, T. (2014). Alimentos Enriquecidos com Vitaminas e Minerais [Food Enriched with Vitamins and Minerals]. Master thesis, University of Lisbon.
Bartali, B., E. Devore, F. Grodstein and J. H. Kang (2014). "Plasma vitamin D levels and cognitive function in aging women: the nurses' health study." J Nutr Health Aging **18**(4): 400-406.
Bartke, A. (2003). "Can growth hormone (GH) accelerate aging? Evidence from GH-transgenic mice." Neuroendocrinology **78**(4): 210-216.
Bartke, A. (2019). "Growth Hormone and Aging: Updated Review." World J Mens Health **37**(1): 19-30.
Bartke, A., E. O. List and J. J. Kopchick (2016). "The somatotropic axis and aging: Benefits of endocrine defects." Growth Horm IGF Res **27**: 41-45.
Bartres-Faz, D. and E. M. Arenaza-Urquijo (2011). "Structural and functional imaging correlates of cognitive and brain reserve hypotheses in healthy and pathological aging." Brain Topogr **24**(3-4): 340-357.
Barzilai, N., D. M. Huffman, R. H. Muzumdar and A. Bartke (2012). "The critical role of metabolic pathways in aging." Diabetes **61**(6): 1315-1322.
Bauer, M., A. Berghofer, T. Bschor, A. Baumgartner, U. Kiesslinger, R. Hellweg, M. Adli, C. Baethge and B. Muller-Oerlinghausen (2002). "Supraphysiological doses of L-thyroxine in the maintenance treatment of prophylaxis-resistant affective disorders." Neuropsychopharmacology **27**(4): 620-628.
Bauer, M., D. H. Silverman, F. Schlagenhauf, E. D. London, C. L. Geist, K. van Herle, N. Rasgon, D. Martinez, K. Miller, A. van Herle, S. M. Berman, M. E. Phelps and P. C. Whybrow (2009). "Brain glucose metabolism in hypothyroidism: a positron emission tomography study before and after thyroid hormone replacement therapy." J Clin Endocrinol Metab **94**(8): 2922-2929.
Baynes, J. W. (2014). Aging. Medical Biochemistry. Elsevier.

Beck, K. D., L. Powell-Braxton, H. R. Widmer, J. Valverde and F. Hefti (1995). "Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons." Neuron **14**(4): 717-730.
Beilfuss, J., V. Berg, M. Sneve, R. Jorde and E. Kamycheva (2012). "Effects of a 1-year supplementation with cholecalciferol on interleukin-6, tumor necrosis factor-alpha and insulin resistance in overweight and obese subjects." Cytokine **60**(3): 870-874.
Belandia, B., M. J. Latasa, A. Villa and A. Pascual (1998). "Thyroid hormone negatively regulates the transcriptional activity of the beta-amyloid precursor protein gene." J Biol Chem **273**(46): 30366-30371.
Beluche, I., I. Carriere, K. Ritchie and M. L. Ancelin (2010). "A prospective study of diurnal cortisol and cognitive function in community-dwelling elderly people." Psychol Med **40**(6): 1039-1049.
Bennett, D. A., S. E. Arnold, M. J. Valenzuela, C. Brayne and J. A. Schneider (2014). "Cognitive and social lifestyle: links with neuropathology and cognition in late life." Acta Neuropathol **127**(1): 137-150.
Bensenor, I. M., P. A. Lotufo, P. R. Menezes and M. Scazufca (2010). "Subclinical hyperthyroidism and dementia: the Sao Paulo Ageing & Health Study (SPAH)." BMC Public Health **10**: 298.
Berardi, A., R. Parasuraman and J. V. Haxby (2001). "Overall vigilance and sustained attention decrements in healthy aging." Exp Aging Res **27**(1): 19-39.
Berbel, P., A. Guadano-Ferraz, A. Angulo and J. Ramon Cerezo (1994). "Role of thyroid hormones in the maturation of interhemispheric connections in rats." Behav Brain Res **64**(1-2): 9-14.
Beresniak, A., A. Medina-Lara, J. P. Auray, A. De Wever, J. C. Praet, R. Tarricone, A. Torbica, D. Dupont, M. Lamure and G. Duru (2015). "Validation of the underlying assumptions of the quality-adjusted life-years outcome: results from the ECHOUTCOME European project." Pharmacoeconomics **33**(1): 61-69.
Berg, A. O., K. N. Jorgensen, M. Nerhus, L. Athanasiu, A. B. Popejoy, F. Bettella, L. C. B. Norbom, T. P. Gurholt, S. R. Dahl, O. A. Andreassen, S. Djurovic, I. Agartz and I. Melle (2018). "Vitamin D levels, brain volume, and genetic architecture in patients with psychosis." PLoS One **13**(8): e0200250.
Bernal, J. (2007). "Thyroid hormone receptors in brain development and function." Nat Clin Pract Endocrinol Metab **3**(3): 249-259.
Bernardes de Jesus, B. and M. A. Blasco (2013). "Telomerase at the intersection of cancer and aging." Trends Genet **29**(9): 513-520.
Berridge, M. J. (2017). "Vitamin D deficiency accelerates ageing and age-related diseases: a novel hypothesis." J Physiol **595**(22): 6825-6836.
Bettencourt, A., D. Boleixa, J. Reis, J. C. Oliveira, D. Mendonca, P. P. Costa, B. M. D. Silva, A. Marinho and A. M. D. Silva (2018). "Serum 25-hydroxyvitamin D levels in a healthy population from the North of Portugal." J Steroid Biochem Mol Biol **175**: 97-101.
Bettencourt, R. and J. A. Serrano (1891). "Un cas de myxœdème (cachexie pachydermique) traité par la greffe hypodermique du corps thyroïde d'un mouton." Compte rendu de la 19eme session de l'Association française our l'avancement des sciences(1890).
Beydoun, M. A., H. A. Beydoun, M. H. Kitner-Triolo, J. S. Kaufman, M. K. Evans and A. B. Zonderman (2013). "Thyroid hormones are associated with cognitive function: moderation by sex, race, and depressive symptoms." J Clin Endocrinol Metab **98**(8): 3470-3481.
Beydoun, M. A., H. A. Beydoun, M. R. Shroff, M. H. Kitner-Triolo and A. B. Zonderman (2012). "Serum leptin, thyroxine and thyroid-stimulating hormone levels interact to affect cognitive function among US adults: evidence from a large representative survey." Neurobiol Aging **33**(8): 1730-1743.

Beydoun, M. A., S. Hossain, M. T. Fanelli-Kuczmarski, H. A. Beydoun, J. A. Canas, M. K. Evans and A. B. Zonderman (2018). "Vitamin D Status and Intakes and Their Association With Cognitive Trajectory in a Longitudinal Study of Urban Adults." J Clin Endocrinol Metab **103**(4): 1654-1668.
Bianco, A. C., A. Dumitrescu, B. Gereben, M. O. Ribeiro, T. L. Fonseca, G. W. Fernandes and B. Bocco (2019). "Paradigms of Dynamic Control of Thyroid Hormone Signaling." Endocr Rev **40**(4): 1000-1047.
Bianco, A. C. and B. W. Kim (2006). "Deiodinases: implications of the local control of thyroid hormone action." J Clin Invest **116**(10): 2571-2579.
Bidlingmaier, M. and P. U. Freda (2010). "Measurement of human growth hormone by immunoassays: current status, unsolved problems and clinical consequences." Growth Horm IGF Res **20**(1): 19-25.
Bidlingmaier, M., N. Friedrich, R. T. Emeny, J. Spranger, O. D. Wolthers, J. Roswall, A. Korner, B. Obermayer-Pietsch, C. Hubener, J. Dahlgren, J. Frystyk, A. F. Pfeiffer, A. Doering, M. Bielohuby, H. Wallaschofski and A. M. Arafat (2014). "Reference intervals for insulin-like growth factor-1 (igf-i) from birth to senescence: results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations." J Clin Endocrinol Metab **99**(5): 1712-1721.
Binkley, N., B. Dawson-Hughes, R. Durazo-Arvizu, M. Thamm, L. Tian, J. M. Merkel, J. C. Jones, G. D. Carter and C. T. Sempos (2017). "Vitamin D measurement standardization: The way out of the chaos." J Steroid Biochem Mol Biol **173**: 117-121.
Bitto, A., C. Lerner, C. Torres, M. Roell, M. Malaguti, V. Perez, A. Lorenzini, S. Hrelia, Y. Ikeno, M. E. Matzko, R. McCarter and C. Sell (2010). "Long-term IGF-I exposure decreases autophagy and cell viability." PLoS One **5**(9): e12592.
Blackburn, E. H., C. W. Greider and J. W. Szostak (2006). "Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging." Nat Med **12**(10): 1133-1138.
Blackman, M. R., J. D. Sorkin, T. Munzer, M. F. Bellantoni, J. Busby-Whitehead, T. E. Stevens, J. Jayme, K. G. O'Connor, C. Christmas, J. D. Tobin, K. J. Stewart, E. Cottrell, C. St Clair, K. M. Pabst and S. M. Harman (2002). "Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial." JAMA **288**(18): 2282-2292.
Blasco, M. A. (2007). "Telomere length, stem cells and aging." Nat Chem Biol **3**(10): 640-649.
Bloom, G. S. (2014). "Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis." JAMA Neurol **71**(4): 505-508.
Bloss, E. B., W. G. Janssen, B. S. McEwen and J. H. Morrison (2010). "Interactive effects of stress and aging on structural plasticity in the prefrontal cortex." J Neurosci **30**(19): 6726-6731.
Blumenthal, H. T. and I. B. Perlstein (1987). "The aging thyroid. I. A description of lesions and an analysis of their age and sex distribution." J Am Geriatr Soc **35**(9): 843-854.
Bodnoff, S. R., A. G. Humphreys, J. C. Lehman, D. M. Diamond, G. M. Rose and M. J. Meaney (1995). "Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats." J Neurosci **15**(1 Pt 1): 61-69.
Bokov, A., A. Chaudhuri and A. Richardson (2004). "The role of oxidative damage and stress in aging." Mech Ageing Dev **125**(10-11): 811-826.
Boldrini, M., C. A. Fulmore, A. N. Tartt, L. R. Simeon, I. Pavlova, V. Poposka, G. B. Rosoklija, A. Stankov, V. Arango, A. J. Dwork, R. Hen and J. J. Mann (2018). "Human Hippocampal Neurogenesis Persists throughout Aging." Cell Stem Cell **22**(4): 589-599 e585.
Bondy, C., H. Werner, C. T. Roberts, Jr. and D. LeRoith (1992). "Cellular pattern of type-I insulin-like growth factor receptor gene expression during maturation of the rat brain: comparison with insulin-like growth factors I and II." Neuroscience **46**(4): 909-923.

Bondy, C. A., M. A. Bach and W.-H. Lee (1992). "Mapping of brain insulin and insulin-like growth factor receptor gene expression by in situ hybridization." Neuroprotocols **1**(3): 240-249.
Bondy, C. A. and W. H. Lee (1993). "Patterns of insulin-like growth factor and IGF receptor gene expression in the brain. Functional implications." Ann N Y Acad Sci **692**: 33-43.
Bono, G., R. Fancellu, F. Blandini, G. Santoro and M. Mauri (2004). "Cognitive and affective status in mild hypothyroidism and interactions with L-thyroxine treatment." Acta Neurol Scand **110**(1): 59-66.
Boonekamp, J. J., M. J. Simons, L. Hemerik and S. Verhulst (2013). "Telomere length behaves as biomarker of somatic redundancy rather than biological age." Aging Cell **12**(2): 330-332.
Booth, T., I. J. Deary and J. M. Starr (2013). "Thyroid stimulating hormone, free thyroxine and cognitive ability in old age: the Lothian Birth Cohort Study 1936." Psychoneuroendocrinology **38**(4): 597-601.
Born, J., I. Ditschuneit, M. Schreiber, C. Dodt and H. L. Fehm (1995). "Effects of age and gender on pituitary-adrenocortical responsiveness in humans." Eur J Endocrinol **132**(6): 705-711.
Botella-Carretero, J. I., J. M. Galan, C. Caballero, J. Sancho and H. F. Escobar-Morreale (2003). "Quality of life and psychometric functionality in patients with differentiated thyroid carcinoma." Endocr Relat Cancer **10**(4): 601-610.
Bouillon, R. (2016). Chapter 59 - Vitamin D: From Photosynthesis, Metabolism, and Action to Clinical Applications∗ A2 - Jameson, J. Larry. Endocrinology: Adult and Pediatric (Seventh Edition). L. J. D. Groot, D. M. d. Kretser, L. C. Giudice et al. Philadelphia, W.B. Saunders**:** 1018-1037.e1017.
Bouillon, R. (2016). Vitamin D: From Photosynthesis, Metabolism, and Action to Clinical Applications. Endocrinology: Adult and Pediatric (Seventh Edition). L. J. D. Groot, Elsevier Inc.**:** 1018-1037.
Bouillon, R. (2017). "Comparative analysis of nutritional guidelines for vitamin D." Nat Rev Endocrinol **13**(8): 466-479.
Bouillon, R., G. Carmeliet, L. Verlinden, E. van Etten, A. Verstuyf, H. F. Luderer, L. Lieben, C. Mathieu and M. Demay (2008). "Vitamin D and human health: lessons from vitamin D receptor null mice." Endocr Rev **29**(6): 726-776.
Breitberg, A., W. C. Drevets, S. E. Wood, L. Mah, J. Schulkin, B. J. Sahakian and K. Erickson (2013). "Hydrocortisone infusion exerts dose- and sex-dependent effects on attention to emotional stimuli." Brain Cogn **81**(2): 247-255.
Bremner, A. P., P. Feddema, P. J. Leedman, S. J. Brown, J. P. Beilby, E. M. Lim, S. G. Wilson, P. C. O'Leary and J. P. Walsh (2012). "Age-related changes in thyroid function: a longitudinal study of a community-based cohort." J Clin Endocrinol Metab **97**(5): 1554-1562.
Brent, G. A. (2000). "Tissue-specific actions of thyroid hormone: insights from animal models." Rev Endocr Metab Disord **1**(1-2): 27-33.
Brent, G. A. (2012). "Mechanisms of thyroid hormone action." J Clin Invest **122**(9): 3035-3043.
Briones, T. L. and H. Darwish (2012). "Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden." J Neuroinflammation **9**: 244.
Brockmann, K., A. M. Dumitrescu, T. T. Best, F. Hanefeld and S. Refetoff (2005). "X-linked paroxysmal dyskinesia and severe global retardation caused by defective MCT8 gene." J Neurol **252**(6): 663-666.
Brooks, A. J., J. W. Wooh, K. A. Tunny and M. J. Waters (2008). "Growth hormone receptor; mechanism of action." Int J Biochem Cell Biol **40**(10): 1984-1989.
Brown-Sequard, C. E. (1889). "Du role physiologique et therapeutique d'un suc extrait de testicules d'animaux d'apres nombre de faits observes chez l'homme (The physiological and therapeutic role

of animal testicular extract based on several experiments in man)." Arch. Physiol. Norm. Pathol.(1): 739-746.
Brown, E. S., J. W. D, A. Frol, L. Bobadilla, D. A. Khan, M. Hanczyc, A. J. Rush, J. Fleckenstein, E. Babcock and C. M. Cullum (2004). "Hippocampal volume, spectroscopy, cognition, and mood in patients receiving corticosteroid therapy." Biol Psychiatry **55**(5): 538-545.
Brown, E. S., H. Jeon-Slaughter, H. Lu, R. Jamadar, S. Issac, M. Shad, D. Denniston, C. Tamminga, A. Nakamura and B. P. Thomas (2015). "Hippocampal volume in healthy controls given 3-day stress doses of hydrocortisone." Neuropsychopharmacology **40**(5): 1216-1221.
Brown, J., C. M. Cooper-Kuhn, G. Kempermann, H. Van Praag, J. Winkler, F. H. Gage and H. G. Kuhn (2003). "Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis." Eur J Neurosci **17**(10): 2042-2046.
Brown, J., A. Ignatius, M. Amling and F. Barvencik (2013). "New perspectives on vitamin D sources in Germany based on a novel mathematical bottom-up model of 25(OH)D serum concentrations." Eur J Nutr **52**(7): 1733-1742.
Brunn, J., U. Block, G. Ruf, I. Bos, W. P. Kunze and P. C. Scriba (1981). "[Volumetric analysis of thyroid lobes by real-time ultrasound (author's transl)]." Dtsch Med Wochenschr **106**(41): 1338-1340.
Buchanan, T. W., S. Kern, J. S. Allen, D. Tranel and C. Kirschbaum (2004). "Circadian regulation of cortisol after hippocampal damage in humans." Biol Psychiatry **56**(9): 651-656.
Buchanan, T. W. and W. R. Lovallo (2001). "Enhanced memory for emotional material following stress-level cortisol treatment in humans." Psychoneuroendocrinology **26**(3): 307-317.
Buell, J. S., T. M. Scott, B. Dawson-Hughes, G. E. Dallal, I. H. Rosenberg, M. F. Folstein and K. L. Tucker (2009). "Vitamin D is associated with cognitive function in elders receiving home health services." J Gerontol A Biol Sci Med Sci **64**(8): 888-895.
Bukoski, R. D., P. DeWan and D. A. McCarron (1989). "1,25 (OH)2 vitamin D3 modifies growth and contractile function of vascular smooth muscle of spontaneously hypertensive rats." Am J Hypertens **2**(7): 553-556.
Burch, W. (2011). "Using bedtime (PM) and early morning (AM) urine cortisol/creatinine ratios to evaluate pituitary-adrenal function in an office practice." Endocr Pract **17**(4): 591-597.
Burek, C. L. and N. R. Rose (2008). "Autoimmune thyroiditis and ROS." Autoimmun Rev **7**(7): 530-537.
Busch, R. M. and J. S. Chapin (2008). "Review of normative data for common screening measures used to evaluate cognitive functioning in elderly individuals." Clin Neuropsychol **22**(4): 620-650.
Byrne, J. H., M. Voogt, K. M. Turner, D. W. Eyles, J. J. McGrath and T. H. Burne (2013). "The impact of adult vitamin D deficiency on behaviour and brain function in male Sprague-Dawley rats." PLoS One **8**(8): e71593.
Caiafa, P. and M. Zampieri (2005). "DNA methylation and chromatin structure: the puzzling CpG islands." J Cell Biochem **94**(2): 257-265.
Calvo, R., M. J. Obregon, C. Ruiz de Ona, F. Escobar del Rey and G. Morreale de Escobar (1990). "Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain." J Clin Invest **86**(3): 889-899.
Cameron, H. A. and E. Gould (1994). "Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus." Neuroscience **61**(2): 203-209.
Cao, X., F. Kambe, L. C. Moeller, S. Refetoff and H. Seo (2005). "Thyroid hormone induces rapid activation of Akt/protein kinase B-mammalian target of rapamycin-p70S6K cascade through phosphatidylinositol 3-kinase in human fibroblasts." Mol Endocrinol **19**(1): 102-112.

Cappola, A. R., A. M. Arnold, K. Wulczyn, M. Carlson, J. Robbins and B. M. Psaty (2015). "Thyroid function in the euthyroid range and adverse outcomes in older adults." J Clin Endocrinol Metab **100**(3): 1088-1096.
Cappon, J. P., E. Ipp, J. A. Brasel and D. M. Cooper (1993). "Acute effects of high fat and high glucose meals on the growth hormone response to exercise." J Clin Endocrinol Metab **76**(6): 1418-1422.
Carson, M. J., R. R. Behringer, R. L. Brinster and F. A. McMorris (1993). "Insulin-like growth factor I increases brain growth and central nervous system myelination in transgenic mice." Neuron **10**(4): 729-740.
Carvalho, A. C., N. Correia Santos, C. Cunha, C. Portugal-Nunes, J. Cotter, J. A. Palha and N. Sousa (2014). "Valores de TSH e hormonas tiroideias numa amostra de base populacional do Norte de Portugal [TSH and thyroid hormone values in a population-based sample from Northern Portugal]." Rev Endocr Metab Disord **8**(2): 108-109.
Casals, G., L. Foj and M. J. de Osaba (2011). "Day-to-day variation of late-night salivary cortisol in healthy voluntaries." Clin Biochem **44**(8-9): 665-668.
Casanueva, F. F., L. Villanueva, J. A. Cabranes, J. Cabezas-Cerrato and A. Fernandez-Cruz (1984). "Cholinergic mediation of growth hormone secretion elicited by arginine, clonidine, and physical exercise in man." J Clin Endocrinol Metab **59**(3): 526-530.
Cashman, K. D., K. G. Dowling, Z. Skrabakova, M. Gonzalez-Gross, J. Valtuena, S. De Henauw, L. Moreno, C. T. Damsgaard, K. F. Michaelsen, C. Molgaard, R. Jorde, G. Grimnes, G. Moschonis, C. Mavrogianni, Y. Manios, M. Thamm, G. B. Mensink, M. Rabenberg, M. A. Busch, L. Cox, S. Meadows, G. Goldberg, A. Prentice, J. M. Dekker, G. Nijpels, S. Pilz, K. M. Swart, N. M. van Schoor, P. Lips, G. Eiriksdottir, V. Gudnason, M. F. Cotch, S. Koskinen, C. Lamberg-Allardt, R. A. Durazo-Arvizu, C. T. Sempos and M. Kiely (2016). "Vitamin D deficiency in Europe: pandemic?" Am J Clin Nutr **103**(4): 1033-1044.
Castellano, J. M. (2019). "Blood-Based Therapies to Combat Aging." Gerontology **65**(1): 84-89.
Castiglione, D., A. Platania, A. Conti, M. Falla, M. D'Urso and M. Marranzano (2018). "Dietary Micronutrient and Mineral Intake in the Mediterranean Healthy Eating, Ageing, and Lifestyle (MEAL) Study." Antioxidants (Basel) **7**(7).
Castle, M., N. Fiedler, L. C. Pop, S. J. Schneider, Y. Schlussel, D. Sukumar, L. Hao and S. A. Shapses (2019). "Three Doses of Vitamin D and Cognitive Outcomes in Older Women: A Double-Blind Randomized Controlled Trial." J Gerontol A Biol Sci Med Sci.
CDC. (2017). "Centers for Disease Control and Prevention; EQUIP: Ensuring the Quality of Urinary Iodine Procedures." November 2019, from https://www.cdc.gov/labstandards/equip.html.
Ceresini, G., F. Lauretani, M. Maggio, G. P. Ceda, S. Morganti, E. Usberti, C. Chezzi, R. Valcavi, S. Bandinelli, J. M. Guralnik, A. R. Cappola, G. Valenti and L. Ferrucci (2009). "Thyroid function abnormalities and cognitive impairment in elderly people: results of the Invecchiare in Chianti study." J Am Geriatr Soc **57**(1): 89-93.
Cerqueira, J. J., C. Catania, I. Sotiropoulos, M. Schubert, R. Kalisch, O. F. Almeida, D. P. Auer and N. Sousa (2005). "Corticosteroid status influences the volume of the rat cingulate cortex - a magnetic resonance imaging study." J Psychiatr Res **39**(5): 451-460.
Cerqueira, J. J., F. Mailliet, O. F. Almeida, T. M. Jay and N. Sousa (2007). "The prefrontal cortex as a key target of the maladaptive response to stress." J Neurosci **27**(11): 2781-2787.
Cerqueira, J. J., J. M. Pego, R. Taipa, J. M. Bessa, O. F. Almeida and N. Sousa (2005). "Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors." J Neurosci **25**(34): 7792-7800.
Chaker, L., C. Baumgartner, W. P. den Elzen, M. A. Ikram, M. R. Blum, T. H. Collet, S. J. Bakker, A. Dehghan, C. Drechsler, R. N. Luben, A. Hofman, M. L. Portegies, M. Medici, G. Iervasi, D. J.

Stott, I. Ford, A. Bremner, C. Wanner, L. Ferrucci, A. B. Newman, R. P. Dullaart, J. A. Sgarbi, G. Ceresini, R. M. Maciel, R. G. Westendorp, J. W. Jukema, M. Imaizumi, J. A. Franklyn, D. C. Bauer, J. P. Walsh, S. Razvi, K. T. Khaw, A. R. Cappola, H. Volzke, O. H. Franco, J. Gussekloo, N. Rodondi, R. P. Peeters and C. Thyroid Studies (2015). "Subclinical Hypothyroidism and the Risk of Stroke Events and Fatal Stroke: An Individual Participant Data Analysis." J Clin Endocrinol Metab **100**(6): 2181-2191.

Chaker, L., L. G. M. Cremers, T. I. M. Korevaar, M. de Groot, A. Dehghan, O. H. Franco, W. J. Niessen, M. A. Ikram, R. P. Peeters and M. W. Vernooij (2018). "Age-dependent association of thyroid function with brain morphology and microstructural organization: evidence from brain imaging." Neurobiol Aging **61**: 44-51.

Chang, F. E., W. G. Dodds, M. Sullivan, M. H. Kim and W. B. Malarkey (1986). "The acute effects of exercise on prolactin and growth hormone secretion: comparison between sedentary women and women runners with normal and abnormal menstrual cycles." J Clin Endocrinol Metab **62**(3): 551-556.

Charlton, B. G. (1990). "Adrenal cortical innervation and glucocorticoid secretion." J Endocrinol **126**(1): 5-8.

Chatzitomaris, A., R. Hoermann, J. E. Midgley, S. Hering, A. Urban, B. Dietrich, A. Abood, H. H. Klein and J. W. Dietrich (2017). "Thyroid Allostasis-Adaptive Responses of Thyrotropic Feedback Control to Conditions of Strain, Stress, and Developmental Programming." Front Endocrinol (Lausanne) **8**: 163.

Chen, K. H., L. Y. Chuah, S. K. Sim and M. W. Chee (2010). "Hippocampal region-specific contributions to memory performance in normal elderly." Brain Cogn **72**(3): 400-407.

Chen, Y. and T. D. Pham (2013). "Sample entropy and regularity dimension in complexity analysis of cortical surface structure in early Alzheimer's disease and aging." J Neurosci Methods **215**(2): 210-217.

Chen, Z. P. and B. S. Hetzel (2010). "Cretinism revisited." Best Pract Res Clin Endocrinol Metab **24**(1): 39-50.

Cheng, C. M., R. F. Mervis, S. L. Niu, N. Salem, Jr., L. A. Witters, V. Tseng, R. Reinhardt and C. A. Bondy (2003). "Insulin-like growth factor 1 is essential for normal dendritic growth." J Neurosci Res **73**(1): 1-9.

Cheng, S. Y. (2000). "Multiple mechanisms for regulation of the transcriptional activity of thyroid hormone receptors." Rev Endocr Metab Disord **1**(1-2): 9-18.

Cheng, S. Y., J. L. Leonard and P. J. Davis (2010). "Molecular aspects of thyroid hormone actions." Endocr Rev **31**(2): 139-170.

Choi, H. J., M. S. Byun, D. Yi, B. K. Sohn, J. H. Lee, J. Y. Lee, Y. K. Kim, D. Y. Lee and K. R. Group (2017). "Associations of thyroid hormone serum levels with in-vivo Alzheimer's disease pathologies." Alzheimers Res Ther **9**(1): 64.

Choufoer, J. C., M. Vanrhijn and A. Querido (1965). "Endemic Goiter in Western New Guinea. Ii. Clinical Picture, Incidence and Pathogenesis of Endemic Cretinism." J Clin Endocrinol Metab **25**: 385-402.

Chumas, P., B. Condon, D. Oluoch-Olunya, S. Griffiths, D. Hadley and G. Teasdale (1997). "Early changes in peritumorous oedema and contralateral white matter after dexamethasone: a study using proton magnetic resonance spectroscopy." J Neurol Neurosurg Psychiatry **62**(6): 590-595.

Clemmons, D. R. (2011). "Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays." Clin Chem **57**(4): 555-559.

Clemmons, D. R. (2012). "Metabolic actions of insulin-like growth factor-I in normal physiology and diabetes." Endocrinol Metab Clin North Am **41**(2): 425-443, vii-viii.

Clemmons, D. R. (2018). "Role of IGF-binding proteins in regulating IGF responses to changes in metabolism." J Mol Endocrinol **61**(1): T139-T169.
Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences. New Jersey, Lawrence Erlbaum Associates Publishers.
Collette, F., M. Van der Linden, S. Laureys, G. Delfiore, C. Degueldre, A. Luxen and E. Salmon (2005). "Exploring the unity and diversity of the neural substrates of executive functioning." Hum Brain Mapp **25**(4): 409-423.
Collier, T. J., J. Lipton, B. F. Daley, S. Palfi, Y. Chu, C. Sortwell, R. A. Bakay, J. R. Sladek, Jr. and J. H. Kordower (2007). "Aging-related changes in the nigrostriatal dopamine system and the response to MPTP in nonhuman primates: diminished compensatory mechanisms as a prelude to parkinsonism." Neurobiol Dis **26**(1): 56-65.
Colman, R. J., R. M. Anderson, S. C. Johnson, E. K. Kastman, K. J. Kosmatka, T. M. Beasley, D. B. Allison, C. Cruzen, H. A. Simmons, J. W. Kemnitz and R. Weindruch (2009). "Caloric restriction delays disease onset and mortality in rhesus monkeys." Science **325**(5937): 201-204.
Comijs, H. C., L. Gerritsen, B. W. Penninx, M. A. Bremmer, D. J. Deeg and M. I. Geerlings (2010). "The association between serum cortisol and cognitive decline in older persons." Am J Geriatr Psychiatry **18**(1): 42-50.
Consiglio, M., M. Viano, S. Casarin, C. Castagnoli, G. Pescarmona and F. Silvagno (2015). "Mitochondrial and lipogenic effects of vitamin D on differentiating and proliferating human keratinocytes." Exp Dermatol **24**(10): 748-753.
Constant, E. L., S. Adam, X. Seron, R. Bruyer, A. Seghers and C. Daumerie (2005). "Anxiety and depression, attention, and executive functions in hypothyroidism." J Int Neuropsychol Soc **11**(5): 535-544.
Constant, E. L., A. G. de Volder, A. Ivanoiu, A. Bol, D. Labar, A. Seghers, G. Cosnard, J. Melin and C. Daumerie (2001). "Cerebral blood flow and glucose metabolism in hypothyroidism: a positron emission tomography study." J Clin Endocrinol Metab **86**(8): 3864-3870.
Cook, S. E., R. D. Nebes, E. M. Halligan, L. A. Burmeister, J. A. Saxton, M. Ganguli, M. B. Fukui, C. C. Meltzer, R. L. Williams and S. T. DeKosky (2002). "Memory Impairment in Elderly Individuals With a Mildly Elevated Serum TSH: The Role of Processing Resources, Depression and Cerebrovascular Disease." Aging, Neuropsychology, and Cognition **9**(3): 175-183.
Cooke, G. E., S. Mullally, N. Correia, S. M. O'Mara and J. Gibney (2014). "Hippocampal volume is decreased in adults with hypothyroidism." Thyroid **24**(3): 433-440.
Corcuff, J. B., A. Tabarin, M. Rashedi, M. Duclos, P. Roger and D. Ducassou (1998). "Overnight urinary free cortisol determination: a screening test for the diagnosis of Cushing's syndrome." Clin Endocrinol (Oxf) **48**(4): 503-508.
Cornelisse, S., M. Joels and T. Smeets (2011). "A randomized trial on mineralocorticoid receptor blockade in men: effects on stress responses, selective attention, and memory." Neuropsychopharmacology **36**(13): 2720-2728.
Corpas, E., S. M. Harman and M. R. Blackman (1993). "Human growth hormone and human aging." Endocr Rev **14**(1): 20-39.
Corsonello, A., A. Montesanto, M. Berardelli, F. De Rango, S. Dato, V. Mari, B. Mazzei, F. Lattanzio and G. Passarino (2010). "A cross-section analysis of FT3 age-related changes in a group of old and oldest-old subjects, including centenarians' relatives, shows that a down-regulated thyroid function has a familial component and is related to longevity." Age Ageing **39**(6): 723-727.
Costa, P. S., N. C. Santos, P. Cunha, J. A. Palha and N. Sousa (2013). "The use of bayesian latent class cluster models to classify patterns of cognitive performance in healthy ageing." PLoS One **8**(8): e71940.

Costeira, M. J., P. Oliveira, S. Ares, S. Roque, G. M. de Escobar and J. A. Palha (2010). "Parameters of thyroid function throughout and after pregnancy in an iodine-deficient population." Thyroid **20**(9): 995-1001.
Costeira, M. J., P. Oliveira, N. C. Santos, S. Ares, B. Saenz-Rico, G. M. de Escobar and J. A. Palha (2011). "Psychomotor development of children from an iodine-deficient region." J Pediatr **159**(3): 447-453.
Crimmins, E. M. and H. Beltran-Sanchez (2011). "Mortality and morbidity trends: is there compression of morbidity?" J Gerontol B Psychol Sci Soc Sci **66**(1): 75-86.
Crosson, B., H. Benefield, M. A. Cato, J. R. Sadek, A. B. Moore, C. E. Wierenga, K. Gopinath, D. Soltysik, R. M. Bauer, E. J. Auerbach, D. Gokcay, C. M. Leonard and R. W. Briggs (2003). "Left and right basal ganglia and frontal activity during language generation: contributions to lexical, semantic, and phonological processes." J Int Neuropsychol Soc **9**(7): 1061-1077.
Crowe, S. F. (1998). "Decrease in performance on the verbal fluency test as a function of time: evaluation in a young healthy sample." J Clin Exp Neuropsychol **20**(3): 391-401.
Cuervo, A. M. (2008). "Autophagy and aging: keeping that old broom working." Trends Genet **24**(12): 604-612.
Cummings, J. L. and D. F. Benson (1983). Dementia, a clinical approach. Boston, Butterworths.
D'Acquisto, F., M. Perretti and R. J. Flower (2008). "Annexin-A1: a pivotal regulator of the innate and adaptive immune systems." Br J Pharmacol **155**(2): 152-169.
D'Ercole, A. J., G. T. Applewhite and L. E. Underwood (1980). "Evidence that somatomedin is synthesized by multiple tissues in the fetus." Dev Biol **75**(2): 315-328.
D. Sarapura, V. and M. H. Samuel (2017). Thyroid-Stimulating Hormone. The Pituitary, 4th edition, Elsevier**:** 163-201.
David Norris and J. Carr (2013). Vertebrate Endocrinology, 4th edition, Academic Press.
Davidson, D. J., R. T. Zacks and C. C. Williams (2003). "Stroop interference, practice, and aging." Neuropsychol Dev Cogn B Aging Neuropsychol Cogn **10**(2): 85-98.
Davis, F. B., V. Cody, P. J. Davis, L. J. Borzynski and S. D. Blas (1983). "Stimulation by thyroid hormone analogues of red blood cell Ca2+-ATPase activity in vitro. Correlations between hormone structure and biological activity in a human cell system." J Biol Chem **258**(20): 12373-12377.
Davis, P. J., F. Goglia and J. L. Leonard (2016). "Nongenomic actions of thyroid hormone." Nat Rev Endocrinol **12**(2): 111-121.
Davis, S. R., I. Lambrinoudaki, M. Lumsden, G. D. Mishra, L. Pal, M. Rees, N. Santoro and T. Simoncini (2015). "Menopause." Nature Reviews Disease Primers **1**: 15004.
de Escobar, G. M., M. J. Obregon and F. E. del Rey (2004). "Maternal thyroid hormones early in pregnancy and fetal brain development." Best Pract Res Clin Endocrinol Metab **18**(2): 225-248.
de Jong, F. J., T. den Heijer, T. J. Visser, Y. B. de Rijke, H. A. Drexhage, A. Hofman and M. M. Breteler (2006). "Thyroid hormones, dementia, and atrophy of the medial temporal lobe." J Clin Endocrinol Metab **91**(7): 2569-2573.
de Jong, F. J., K. Masaki, H. Chen, A. T. Remaley, M. M. Breteler, H. Petrovitch, L. R. White and L. J. Launer (2009). "Thyroid function, the risk of dementia and neuropathologic changes: the Honolulu-Asia aging study." Neurobiol Aging **30**(4): 600-606.
de Kloet, E. R., M. Joels and F. Holsboer (2005). "Stress and the brain: from adaptation to disease." Nat Rev Neurosci **6**(6): 463-475.
de Kloet, E. R., O. C. Meijer, A. F. de Nicola, R. H. de Rijk and M. Joels (2018). "Importance of the brain corticosteroid receptor balance in metaplasticity, cognitive performance and neuro-inflammation." Front Neuroendocrinol **49**: 124-145.

de Mooij, S. M. M., R. N. A. Henson, L. J. Waldorp and R. A. Kievit (2018). "Age differentiation within grey matter, white matter and between memory and white matter in an adult lifespan cohort." The Journal of Neuroscience.
Dean, A. J., M. A. Bellgrove, T. Hall, W. M. Phan, D. W. Eyles, D. Kvaskoff and J. J. McGrath (2011). "Effects of vitamin D supplementation on cognitive and emotional functioning in young adults–a randomised controlled trial." PLoS One **6**(11): e25966.
Dean, D. S. and H. Gharib (2008). "Epidemiology of thyroid nodules." Best Pract Res Clin Endocrinol Metab **22**(6): 901-911.
Deeks, S. G. (2011). "HIV infection, inflammation, immunosenescence, and aging." Annu Rev Med **62**: 141-155.
Dehkhoda, F., C. M. M. Lee, J. Medina and A. J. Brooks (2018). "The Growth Hormone Receptor: Mechanism of Receptor Activation, Cell Signaling, and Physiological Aspects." Front Endocrinol (Lausanne) **9**: 35.
Deijen, J. B., H. de Boer, G. J. Blok and E. A. van der Veen (1996). "Cognitive impairments and mood disturbances in growth hormone deficient men." Psychoneuroendocrinology **21**(3): 313-322.
DeLong, G. R., J. B. Stanbury and R. Fierro-Benitez (1985). "Neurological signs in congenital iodine-deficiency disorder (endemic cretinism)." Dev Med Child Neurol **27**(3): 317-324.
DeLuca, H. F. (1988). "The vitamin D story: a collaborative effort of basic science and clinical medicine." FASEB J **2**(3): 224-236.
Denham, M. J. and E. J. Wills (1980). "A clinico-pathological survey of thyroid glands in old age." Gerontology **26**(3): 160-166.
Dentice, M., A. Marsili, A. Zavacki, P. R. Larsen and D. Salvatore (2013). "The deiodinases and the control of intracellular thyroid hormone signaling during cellular differentiation." Biochim Biophys Acta **1830**(7): 3937-3945.
Devesa, J., C. Almenglo and P. Devesa (2016). "Multiple Effects of Growth Hormone in the Body: Is it Really the Hormone for Growth?" Clin Med Insights Endocrinol Diabetes **9**: 47-71.
Devinsky, O., M. J. Morrell and B. A. Vogt (1995). "Contributions of anterior cingulate cortex to behaviour." Brain **118 (Pt 1)**: 279-306.
Dhesi, J. K., S. H. Jackson, L. M. Bearne, C. Moniz, M. V. Hurley, C. G. Swift and T. J. Allain (2004). "Vitamin D supplementation improves neuromuscular function in older people who fall." Age Ageing **33**(6): 589-595.
Diamond, D. M., M. C. Bennett, M. Fleshner and G. M. Rose (1992). "Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation." Hippocampus **2**(4): 421-430.
Dierolf, A. M., L. E. Arlt, K. Roelofs, M. Kolsch, M. J. Hulsemann, H. Schachinger and E. Naumann (2016). "Effects of basal and acute cortisol on cognitive flexibility in an emotional task switching paradigm in men." Horm Behav **81**: 12-19.
Dietrich, J. W., J. E. M. Midgley and R. Hoermann (2018). "Editorial: "Homeostasis and Allostasis of Thyroid Function"." Front Endocrinol (Lausanne) **9**: 287.
DiLoreto, R. and C. T. Murphy (2015). "The cell biology of aging." Mol Biol Cell **26**(25): 4524-4531.
Direcção-Geral da Saúde , D. (2008). Orientação técnica sobre suplemento de Cálcio e Vitamina D em pessoas idosas. D. d. P. e. C. d. Doença.
Dokoumetzidis, A., A. Iliadis and P. Macheras (2002). "Nonlinear dynamics in clinical pharmacology: the paradigm of cortisol secretion and suppression." Br J Clin Pharmacol **54**(1): 21-29.

Donahue, C. P., K. S. Kosik and T. J. Shors (2006). "Growth hormone is produced within the hippocampus where it responds to age, sex, and stress." Proc Natl Acad Sci U S A **103**(15): 6031-6036.
Donda, A. and T. Lemarchand-Beraud (1989). "Aging alters the activity of 5'-deiodinase in the adenohypophysis, thyroid gland, and liver of the male rat." Endocrinology **124**(3): 1305-1309.
Dong, X., B. Milholland and J. Vijg (2016). "Evidence for a limit to human lifespan." Nature **538**(7624): 257-259.
Doyle, L. (1991). "Myxoedema: some early reports and contributions by British authors, 1873-1898." J R Soc Med **84**(2): 103-106.
Du Puy, R. S., R. K. E. Poortvliet, M. Snel, W. P. J. den Elzen, B. Ballieux, O. M. Dekkers, S. P. Mooijaart and J. Gussekloo (2019). "Associations of Elevated Antithyroperoxidase Antibodies with Thyroid Function, Survival, Functioning, and Depressive Symptoms in the Oldest Old: The Leiden 85-plus Study." Thyroid **29**(9): 1201-1208.
Dubiel, R., L. Callender, C. Dunklin, C. Harper, M. Bennett, L. Kreber, R. Auchus and R. Diaz-Arrastia (2018). "Phase 2 Randomized, Placebo-Controlled Clinical Trial of Recombinant Human Growth Hormone (rhGH) During Rehabilitation From Traumatic Brain Injury." Front Endocrinol (Lausanne) **9**: 520.
Dumitriu, D., J. Hao, Y. Hara, J. Kaufmann, W. G. Janssen, W. Lou, P. R. Rapp and J. H. Morrison (2010). "Selective changes in thin spine density and morphology in monkey prefrontal cortex correlate with aging-related cognitive impairment." J Neurosci **30**(22): 7507-7515.
Dumont, J. E., C. Maenhaut, I. Pirson, M. Baptist and P. P. Roger (1991). "Growth factors controlling the thyroid gland." Baillieres Clin Endocrinol Metab **5**(4): 727-754.
Duron, E., B. Funalot, N. Brunel, J. Coste, L. Quinquis, C. Viollet, J. Belmin, P. Jouanny, F. Pasquier, J. M. Treluyer, J. Epelbaum, Y. le Bouc and O. Hanon (2012). "Insulin-like growth factor-I and insulin-like growth factor binding protein-3 in Alzheimer's disease." J Clin Endocrinol Metab **97**(12): 4673-4681.
Eayrs, J. T. and G. Horn (1955). "The development of cerebral cortex in hypothyroid and starved rats." Anat Rec **121**(1): 53-61.
Eayrs, J. T. and S. H. Taylor (1951). "The effect of thyroid deficiency induced by methyl thiouracil on the maturation of the central nervous system." J Anat **85**(4): 350-358.
Echouffo-Tcheugui, J. B., S. C. Conner, J. J. Himali, P. Maillard, C. S. DeCarli, A. S. Beiser, R. S. Vasan and S. Seshadri (2018). "Circulating cortisol and cognitive and structural brain measures: The Framingham Heart Study." Neurology **91**(21): e1961-e1970.
Economos, A. C. (1979). "A non-Gompertzian paradigm for mortality kinetics of metazoan animals and failure kinetics of manufactured products." AGE **2**(3): 74-76.
Egeland, M., P. A. Zunszain and C. M. Pariante (2015). "Molecular mechanisms in the regulation of adult neurogenesis during stress." Nat Rev Neurosci **16**(4): 189-200.
EHLEIS (2018). EHLEIS Technical report 2018_4.1, European Health and Life Expectancy information system.
Eldred, K. C., S. E. Hadyniak, K. A. Hussey, B. Brenerman, P. W. Zhang, X. Chamling, V. M. Sluch, D. S. Welsbie, S. Hattar, J. Taylor, K. Wahlin, D. J. Zack and R. J. Johnston, Jr. (2018). "Thyroid hormone signaling specifies cone subtypes in human retinal organoids." Science **362**(6411).
Ennis, G. E., S. D. Moffat and C. Hertzog (2016). "The cortisol awakening response and cognition across the adult lifespan." Brain Cogn **105**: 66-77.
Epstein, C. J., G. M. Martin, A. L. Schultz and A. G. Motulsky (1966). "Werner's syndrome a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process." Medicine (Baltimore) **45**(3): 177-221.

Eriksson, M., W. T. Brown, L. B. Gordon, M. W. Glynn, J. Singer, L. Scott, M. R. Erdos, C. M. Robbins, T. Y. Moses, P. Berglund, A. Dutra, E. Pak, S. Durkin, A. B. Csoka, M. Boehnke, T. W. Glover and F. S. Collins (2003). "Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome." Nature **423**(6937): 293-298.
Esfandiari, N. H. and M. Papaleontiou (2017). "Biochemical Testing in Thyroid Disorders." Endocrinol Metab Clin North Am **46**(3): 631-648.
Eurostat (2018). Eurostat database, European Comission.
Evans, H. M. and J. A. Long (1922). "Characteristic Effects upon Growth, Oestrus and Ovulation Induced by the Intraperitoneal Administration of Fresh Anterior Hypophyseal Substance." Proc Natl Acad Sci U S A **8**(3): 38-39.
Evans, P., F. Hucklebridge, C. Loveday and A. Clow (2012). "The cortisol awakening response is related to executive function in older age." Int J Psychophysiol **84**(2): 201-204.
Eyles, D., J. Brown, A. Mackay-Sim, J. McGrath and F. Feron (2003). "Vitamin D3 and brain development." Neuroscience **118**(3): 641-653.
Eyles, D. W., S. Smith, R. Kinobe, M. Hewison and J. J. McGrath (2005). "Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain." J Chem Neuroanat **29**(1): 21-30.
Faggioli, F., T. Wang, J. Vijg and C. Montagna (2012). "Chromosome-specific accumulation of aneuploidy in the aging mouse brain." Hum Mol Genet **21**(24): 5246-5253.
Falleti, M. G., P. Maruff, P. Burman and A. Harris (2006). "The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: a meta-analysis of the current literature." Psychoneuroendocrinology **31**(6): 681-691.
Fama, R., E. V. Sullivan, P. K. Shear, D. A. Cahn-Weiner, L. Marsh, K. O. Lim, J. A. Yesavage, J. R. Tinklenberg and A. Pfefferbaum (2000). "Structural brain correlates of verbal and nonverbal fluency measures in Alzheimer's disease." Neuropsychology **14**(1): 29-40.
Fan, J., B. D. McCandliss, T. Sommer, A. Raz and M. I. Posner (2002). "Testing the efficiency and independence of attentional networks." J Cogn Neurosci **14**(3): 340-347.
Faupel-Badger, J. M., D. Berrigan, R. Ballard-Barbash and N. Potischman (2009). "Anthropometric correlates of insulin-like growth factor 1 (IGF-1) and IGF binding protein-3 (IGFBP-3) levels by race/ethnicity and gender." Ann Epidemiol **19**(12): 841-849.
Faustino, L. C. and T. M. Ortiga-Carvalho (2014). "Thyroid hormone role on cerebellar development and maintenance: a perspective based on transgenic mouse models." Front Endocrinol (Lausanne) **5**: 75.
Fernandes, A. G. (2015). The thyroid in aging : association between thyroid hormones and brain resting state networks in healthy older people. Master in Medicine, University of Minho.
Fernandez, A. M. and I. Torres-Aleman (2012). "The many faces of insulin-like peptide signalling in the brain." Nat Rev Neurosci **13**(4): 225-239.
Feron, F., T. H. Burne, J. Brown, E. Smith, J. J. McGrath, A. Mackay-Sim and D. W. Eyles (2005). "Developmental Vitamin D3 deficiency alters the adult rat brain." Brain Res Bull **65**(2): 141-148.
Ferrari, E., L. Cravello, F. Falvo, L. Barili, S. B. Solerte, M. Fioravanti and F. Magri (2008). "Neuroendocrine features in extreme longevity." Exp Gerontol **43**(2): 88-94.
Flamant, F., K. Gauthier and S. Richard (2017). "Genetic Investigation of Thyroid Hormone Receptor Function in the Developing and Adult Brain." Curr Top Dev Biol **125**: 303-335.
Fletcher, P. C. and R. N. Henson (2001). "Frontal lobes and human memory: insights from functional neuroimaging." Brain **124**(Pt 5): 849-881.
Flores, I., M. L. Cayuela and M. A. Blasco (2005). "Effects of telomerase and telomere length on epidermal stem cell behavior." Science **309**(5738): 1253-1256.

Flurkey, K., J. Papaconstantinou, R. A. Miller and D. E. Harrison (2001). "Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production." Proc Natl Acad Sci U S A **98**(12): 6736-6741.
Fontana, L., L. Partridge and V. D. Longo (2010). "Extending healthy life span–from yeast to humans." Science **328**(5976): 321-326.
Forget, H., A. Lacroix, I. Bourdeau and H. Cohen (2016). "Long-term cognitive effects of glucocorticoid excess in Cushing's syndrome." Psychoneuroendocrinology **65**: 26-33.
Forti, P., V. Olivelli, E. Rietti, B. Maltoni, G. Pirazzoli, R. Gatti, M. G. Gioia and G. Ravaglia (2012). "Serum thyroid-stimulating hormone as a predictor of cognitive impairment in an elderly cohort." Gerontology **58**(1): 41-49.
Franceschi, C., M. Capri, D. Monti, S. Giunta, F. Olivieri, F. Sevini, M. P. Panourgia, L. Invidia, L. Celani, M. Scurti, E. Cevenini, G. C. Castellani and S. Salvioli (2007). "Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans." Mech Ageing Dev **128**(1): 92-105.
Franceschi, C., P. Garagnani, G. Vitale, M. Capri and S. Salvioli (2017). "Inflammaging and 'Garb-aging'." Trends Endocrinol Metab **28**(3): 199-212.
Franceschi, C., R. Ostan, S. Mariotti, D. Monti and G. Vitale (2019). "The Aging Thyroid: A Reappraisal Within the Geroscience Integrated Perspective." Endocr Rev **40**(5): 1250-1270.
Franz, C. E., R. C. O'Brien, R. L. Hauger, S. P. Mendoza, M. S. Panizzon, E. Prom-Wormley, L. J. Eaves, K. Jacobson, M. J. Lyons, S. Lupien, D. Hellhammer, H. Xian and W. S. Kremen (2011). "Cross-sectional and 35-year longitudinal assessment of salivary cortisol and cognitive functioning: the Vietnam Era twin study of aging." Psychoneuroendocrinology **36**(7): 1040-1052.
Frater, J., D. Lie, P. Bartlett and J. J. McGrath (2018). "Insulin-like Growth Factor 1 (IGF-1) as a marker of cognitive decline in normal ageing: A review." Ageing Res Rev **42**: 14-27.
Friedlander, A. L., G. E. Butterfield, S. Moynihan, J. Grillo, M. Pollack, L. Holloway, L. Friedman, J. Yesavage, D. Matthias, S. Lee, R. Marcus and A. R. Hoffman (2001). "One year of insulin-like growth factor I treatment does not affect bone density, body composition, or psychological measures in postmenopausal women." J Clin Endocrinol Metab **86**(4): 1496-1503.
Fries, J. F. (1980). "Aging, natural death, and the compression of morbidity." N Engl J Med **303**(3): 130-135.
Friesema, E. C., S. Ganguly, A. Abdalla, J. E. Manning Fox, A. P. Halestrap and T. J. Visser (2003). "Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter." J Biol Chem **278**(41): 40128-40135.
Frimodt-Moller, K. E., J. R. Mollegaard Jepsen, U. Feldt-Rasmussen and J. Krogh (2019). "Hippocampal Volume, Cognitive Functions, Depression, Anxiety, and Quality of Life in Patients With Cushing Syndrome." J Clin Endocrinol Metab **104**(10): 4563-4577.
Frystyk, J., P. Freda and D. R. Clemmons (2010). "The current status of IGF-I assays–a 2009 update." Growth Horm IGF Res **20**(1): 8-18.
Gaillard, J. M. and C. Bonenfant (2008). "Vertebrate ageing: an evolutionary process with a genetic basis?" Curr Biol **18**(3): R130-131.
Gan, E. H. and S. H. Pearce (2012). "Clinical review: The thyroid in mind: cognitive function and low thyrotropin in older people." J Clin Endocrinol Metab **97**(10): 3438-3449.
Garaschuk, O., H. M. Semchyshyn and V. I. Lushchak (2018). "Healthy brain aging: Interplay between reactive species, inflammation and energy supply." Ageing Res Rev **43**: 26-45.
Garasto, S., A. Montesanto, A. Corsonello, F. Lattanzio, S. Fusco, G. Passarino, V. Prestipino Giarritta and F. Corica (2017). "Thyroid hormones in extreme longevity." Mech Ageing Dev **165**(Pt B): 98-106.

Gardner, M. P., S. Lightman, A. A. Sayer, C. Cooper, R. Cooper, D. Deeg, S. Ebrahim, J. Gallacher, M. Kivimaki, M. Kumari, D. Kuh, R. M. Martin, G. Peeters, Y. Ben-Shlomo and T. Halcyon Study (2013). "Dysregulation of the hypothalamic pituitary adrenal (HPA) axis and physical performance at older ages: an individual participant meta-analysis." Psychoneuroendocrinology **38**(1): 40-49.
Garschall, K. and T. Flatt (2018). "The interplay between immunity and aging in Drosophila." F1000Res **7**: 160.
Gavrilov, L. A. and N. S. Gavrilova (1991). The Biology of Life Span: A Quantitative Approach. New York, Harwood Academic Publishers.
Gavrilov, L. A. and N. S. Gavrilova (2001). "The reliability theory of aging and longevity." J Theor Biol **213**(4): 527-545.
Gavrilov, L. A. and N. S. Gavrilova (2003). "The quest for a general theory of aging and longevity." Sci Aging Knowledge Environ **2003**(28): RE5.
Gavrilov, L. A. and N. S. Gavrilova (2011). "Mortality Measurement at Advanced Ages: A Study of the Social Security Administration Death Master File." N Am Actuar J **15**(3): 432-447.
Geerlings, M. I., S. Sigurdsson, G. Eiriksdottir, M. E. Garcia, T. B. Harris, V. Gudnason and L. J. Launer (2015). "Salivary cortisol, brain volumes, and cognition in community-dwelling elderly without dementia." Neurology **85**(11): 976-983.
George, C., G. Gontier, P. Lacube, J. C. Francois, M. Holzenberger and S. Aid (2017). "The Alzheimer's disease transcriptome mimics the neuroprotective signature of IGF-1 receptor-deficient neurons." Brain **140**(7): 2012-2027.
Gereben, B., A. M. Zavacki, S. Ribich, B. W. Kim, S. A. Huang, W. S. Simonides, A. Zeold and A. C. Bianco (2008). "Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling." Endocr Rev **29**(7): 898-938.
Gereben, B., A. Zeold, M. Dentice, D. Salvatore and A. C. Bianco (2008). "Activation and inactivation of thyroid hormone by deiodinases: local action with general consequences." Cell Mol Life Sci **65**(4): 570-590.
Ghassabian, A., H. El Marroun, R. P. Peeters, V. W. Jaddoe, A. Hofman, F. C. Verhulst, H. Tiemeier and T. White (2014). "Downstream effects of maternal hypothyroxinemia in early pregnancy: nonverbal IQ and brain morphology in school-age children." J Clin Endocrinol Metab **99**(7): 2383-2390.
Gianaros, P. J., J. R. Jennings, L. K. Sheu, P. J. Greer, L. H. Kuller and K. A. Matthews (2007). "Prospective reports of chronic life stress predict decreased grey matter volume in the hippocampus." Neuroimage **35**(2): 795-803.
Gil, A., J. Plaza-Diaz and M. D. Mesa (2018). "Vitamin D: Classic and Novel Actions." Ann Nutr Metab **72**(2): 87-95.
Giustina, A., R. A. Adler, N. Binkley, R. Bouillon, P. R. Ebeling, M. Lazaretti-Castro, C. Marcocci, R. Rizzoli, C. T. Sempos and J. P. Bilezikian (2019). "Controversies in Vitamin D: Summary Statement From an International Conference." J Clin Endocrinol Metab **104**(2): 234-240.
Giustina, A. and J. D. Veldhuis (1998). "Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human." Endocr Rev **19**(6): 717-797.
Gobel, A., M. Gottlich, M. Heldmann, R. Georges, R. Nieberding, B. Rogge, A. Sartorius, G. Brabant and T. F. Munte (2018). "Experimentally induced subclinical hypothyroidism causes decreased functional connectivity of the cuneus: A resting state fMRI study." Psychoneuroendocrinology **102**: 158-163.
Gobel, A., M. Gottlich, M. Heldmann, R. Georges, R. Nieberding, B. Rogge, A. Sartorius, G. Brabant and T. F. Munte (2019). "Experimentally induced subclinical hypothyroidism causes decreased functional connectivity of the cuneus: A resting state fMRI study." Psychoneuroendocrinology **102**: 158-163.

Gobel, A., M. Heldmann, M. Gottlich, A. L. Dirk, G. Brabant and T. F. Munte (2015). "Effect of Experimental Thyrotoxicosis on Brain Gray Matter: A Voxel-Based Morphometry Study." Eur Thyroid J **4**(Suppl 1): 113-118.
Goldberger, A. L., L. A. Amaral, J. M. Hausdorff, P. Ivanov, C. K. Peng and H. E. Stanley (2002). "Fractal dynamics in physiology: alterations with disease and aging." Proc Natl Acad Sci U S A **99 Suppl 1**: 2466-2472.
Gompertz, B. (1825). "On the Nature of the Function Expressive of the Law of Human Mortality, and on a New Mode of Determining the Value of Life Contingencies." Philosophical Transactions of the Royal Society(115): 513-585.
Gong, J., J. Dong, Y. Wang, H. Xu, W. Wei, J. Zhong, W. Liu, Q. Xi and J. Chen (2010). "Developmental iodine deficiency and hypothyroidism impair neural development, up-regulate caveolin-1 and down-regulate synaptophysin in rat hippocampus." J Neuroendocrinol **22**(2): 129-139.
Gontier, G., C. George, Z. Chaker, M. Holzenberger and S. Aid (2015). "Blocking IGF Signaling in Adult Neurons Alleviates Alzheimer's Disease Pathology through Amyloid-beta Clearance." J Neurosci **35**(33): 11500-11513.
Gonzalez-Molero, I., S. Morcillo, S. Valdes, V. Perez-Valero, P. Botas, E. Delgado, D. Hernandez, G. Olveira, G. Rojo, C. Gutierrez-Repiso, E. Rubio-Martin, E. Menendez and F. Soriguer (2011). "Vitamin D deficiency in Spain: a population-based cohort study." Eur J Clin Nutr **65**(3): 321-328.
Goodell, M. A. and T. A. Rando (2015). "Stem cells and healthy aging." Science **350**(6265): 1199-1204.
Goodman, J. H. and M. E. Gilbert (2007). "Modest thyroid hormone insufficiency during development induces a cellular malformation in the corpus callosum: a model of cortical dysplasia." Endocrinology **148**(6): 2593-2597.
Goodwill, A. M., S. Campbell, S. Simpson, Jr., M. Bisignano, C. Chiang, L. Dennerstein and C. Szoeke (2018). "Vitamin D status is associated with executive function a decade later: Data from the Women's Healthy Ageing Project." Maturitas **107**: 56-62.
Goodwill, A. M. and C. Szoeke (2017). "A Systematic Review and Meta-Analysis of The Effect of Low Vitamin D on Cognition." J Am Geriatr Soc **65**(10): 2161-2168.
Gore, A. (2008). Neuroendocrine Systems. Fundamental Neuroscience, 3rd edition. L. Squire, Academic Press**:** 905-930.
Gorgoulis, V. G. and T. D. Halazonetis (2010). "Oncogene-induced senescence: the bright and dark side of the response." Curr Opin Cell Biol **22**(6): 816-827.
Gossard, F., F. Dihl, G. Pelletier, P. M. Dubois and G. Morel (1987). "In situ hybridization to rat brain and pituitary gland of growth hormone cDNA." Neurosci Lett **79**(3): 251-256.
Gottlich, M., M. Heldmann, A. Gobel, A. L. Dirk, G. Brabant and T. F. Munte (2015). "Experimentally induced thyrotoxicosis leads to increased connectivity in temporal lobe structures: a resting state fMRI study." Psychoneuroendocrinology **56**: 100-109.
Gould, E., P. Tanapat, B. S. McEwen, G. Flugge and E. Fuchs (1998). "Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress." Proc Natl Acad Sci U S A **95**(6): 3168-3171.
Grafton, S. T., R. P. Woods and M. Tyszka (1994). "Functional imaging of procedural motor learning: Relating cerebral blood flow with individual subject performance." Hum Brain Mapp **1**(3): 221-234.
Graham, U. M., S. J. Hunter, M. McDonnell, K. R. Mullan and A. B. Atkinson (2013). "A comparison of the use of urinary cortisol to creatinine ratios and nocturnal salivary cortisol in the evaluation of cyclicity in patients with Cushing's syndrome." J Clin Endocrinol Metab **98**(1): E72-76.

Granic, A., T. Aspray, T. Hill, K. Davies, J. Collerton, C. Martin-Ruiz, T. von Zglinicki, T. B. Kirkwood, J. C. Mathers and C. Jagger (2015). "25-hydroxyvitamin D and increased all-cause mortality in very old women: the Newcastle 85+ study." J Intern Med **277**(4): 456-467.
Granic, A., T. R. Hill, T. B. Kirkwood, K. Davies, J. Collerton, C. Martin-Ruiz, T. von Zglinicki, B. K. Saxby, K. A. Wesnes, D. Collerton, J. C. Mathers and C. Jagger (2015). "Serum 25-hydroxyvitamin D and cognitive decline in the very old: the Newcastle 85+ Study." Eur J Neurol **22**(1): 106-115, e106-107.
Green, C. J., J. M. Holly, A. Bayer, M. Fish, S. Ebrahim, J. Gallacher and Y. Ben-Shlomo (2014). "The role of IGF-I, IGF-II, and IGFBP-3 in male cognitive aging and dementia risk: the Caerphilly Prospective Study." J Alzheimers Dis **41**(3): 867-875.
Grigoletto, F., G. Zappala, D. W. Anderson and B. D. Lebowitz (1999). "Norms for the Mini-Mental State Examination in a healthy population." Neurology **53**(2): 315-320.
Groeneweg, S., W. E. Visser and T. J. Visser (2017). "Disorder of thyroid hormone transport into the tissues." Best Pract Res Clin Endocrinol Metab **31**(2): 241-253.
Groves, N. J., J. P. Kesby, D. W. Eyles, J. J. McGrath, A. Mackay-Sim and T. H. Burne (2013). "Adult vitamin D deficiency leads to behavioural and brain neurochemical alterations in C57BL/6J and BALB/c mice." Behav Brain Res **241**: 120-131.
Gubbels Bupp, M. R., T. Potluri, A. L. Fink and S. L. Klein (2018). "The Confluence of Sex Hormones and Aging on Immunity." Front Immunol **9**: 1269.
Gubbi, S., G. F. Quipildor, N. Barzilai, D. M. Huffman and S. Milman (2018). "40 YEARS of IGF1: IGF1: the Jekyll and Hyde of the aging brain." J Mol Endocrinol **61**(1): T171-T185.
Guerreiro, M., Silva, A. P., Botelho,M. A., Leitão, O., Castro-Caldas, A., & Garcia, C (1994). "Adaptação à população portuguesa da tradução do mini mental state examination (MMSE)." Revista Portuguesa de Neurologia **1**(9): 9-10.
Guevara-Aguirre, J., P. Balasubramanian, M. Guevara-Aguirre, M. Wei, F. Madia, C. W. Cheng, D. Hwang, A. Martin-Montalvo, J. Saavedra, S. Ingles, R. de Cabo, P. Cohen and V. D. Longo (2011). "Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans." Sci Transl Med **3**(70): 70ra13.
Guo, C. C., M. L. Gorno-Tempini, B. Gesierich, M. Henry, A. Trujillo, T. Shany-Ur, J. Jovicich, S. D. Robinson, J. H. Kramer, K. P. Rankin, B. L. Miller and W. W. Seeley (2013). "Anterior temporal lobe degeneration produces widespread network-driven dysfunction." Brain **136**(Pt 10): 2979-2991.
Gussekloo, J., E. van Exel, A. J. de Craen, A. E. Meinders, M. Frolich and R. G. Westendorp (2004). "Thyroid status, disability and cognitive function, and survival in old age." JAMA **292**(21): 2591-2599.
Hadlow, N. C., K. M. Rothacker, R. Wardrop, S. J. Brown, E. M. Lim and J. P. Walsh (2013). "The relationship between TSH and free T(4) in a large population is complex and nonlinear and differs by age and sex." J Clin Endocrinol Metab **98**(7): 2936-2943.
Hagenau, T., R. Vest, T. N. Gissel, C. S. Poulsen, M. Erlandsen, L. Mosekilde and P. Vestergaard (2009). "Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: an ecologic meta-regression analysis." Osteoporos Int **20**(1): 133-140.
Hammes, S. R. and P. J. Davis (2015). "Overlapping nongenomic and genomic actions of thyroid hormone and steroids." Best Pract Res Clin Endocrinol Metab **29**(4): 581-593.
Hannan, A. J., S. Servotte, A. Katsnelson, S. Sisodiya, C. Blakemore, M. Squier and Z. Molnar (1999). "Characterization of nodular neuronal heterotopia in children." Brain **122 (Pt 2)**: 219-238.

Hara, Y., M. Punsoni, F. Yuk, C. S. Park, W. G. Janssen, P. R. Rapp and J. H. Morrison (2012). "Synaptic distributions of GluA2 and PKMzeta in the monkey dentate gyrus and their relationships with aging and memory." J Neurosci **32**(21): 7336-7344.
Harington, C. R. and G. Barger (1927). "Chemistry of Thyroxine: Constitution and Synthesis of Thyroxine." Biochem J **21**(1): 169-183.
Harman, D. (1956). "Aging: a theory based on free radical and radiation chemistry." J Gerontol **11**(3): 298-300.
Harries, L. W., D. Hernandez, W. Henley, A. R. Wood, A. C. Holly, R. M. Bradley-Smith, H. Yaghootkar, A. Dutta, A. Murray, T. M. Frayling, J. M. Guralnik, S. Bandinelli, A. Singleton, L. Ferrucci and D. Melzer (2011). "Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing." Aging Cell **10**(5): 868-878.
Harris, M. A., S. R. Cox, C. E. Brett, I. J. Deary and A. M. J. MacLullich (2017). "Cognitive ability across the life course and cortisol levels in older age." Neurobiol Aging **59**: 64-71.
Harrison, D. E., R. Strong, Z. D. Sharp, J. F. Nelson, C. M. Astle, K. Flurkey, N. L. Nadon, J. E. Wilkinson, K. Frenkel, C. S. Carter, M. Pahor, M. A. Javors, E. Fernandez and R. A. Miller (2009). "Rapamycin fed late in life extends lifespan in genetically heterogeneous mice." Nature **460**(7253): 392-395.
Harrison, R. F., M. Debono, M. J. Whitaker, B. G. Keevil, J. Newell-Price and R. J. Ross (2019). "Salivary Cortisone to Estimate Cortisol Exposure and Sampling Frequency Required Based on Serum Cortisol Measurements." J Clin Endocrinol Metab **104**(3): 765-772.
Hartman, M. L., A. C. Faria, M. L. Vance, M. L. Johnson, M. O. Thorner and J. D. Veldhuis (1991). "Temporal structure of in vivo growth hormone secretory events in humans." Am J Physiol **260**(1 Pt 1): E101-110.
Hauger, R. L., K. V. Thrivikraman and P. M. Plotsky (1994). "Age-related alterations of hypothalamic-pituitary-adrenal axis function in male Fischer 344 rats." Endocrinology **134**(3): 1528-1536.
Haussler, M. R., P. W. Jurutka, M. Mizwicki and A. W. Norman (2011). "Vitamin D receptor (VDR)-mediated actions of 1alpha,25(OH)(2)vitamin D(3): genomic and non-genomic mechanisms." Best Pract Res Clin Endocrinol Metab **25**(4): 543-559.
Haussler, M. R., G. K. Whitfield, I. Kaneko, C. A. Haussler, D. Hsieh, J. C. Hsieh and P. W. Jurutka (2013). "Molecular mechanisms of vitamin D action." Calcif Tissue Int **92**(2): 77-98.
Hayflick, L. (2007). "Biological aging is no longer an unsolved problem." Ann N Y Acad Sci **1100**: 1-13.
Hayflick, L. and P. S. Moorhead (1961). "The serial cultivation of human diploid cell strains." Exp Cell Res **25**: 585-621.
Hazem, A., M. B. Elamin, G. Malaga, I. Bancos, Y. Prevost, C. Zeballos-Palacios, E. R. Velasquez, P. J. Erwin, N. Natt, V. M. Montori and M. H. Murad (2011). "The accuracy of diagnostic tests for GH deficiency in adults: a systematic review and meta-analysis." Eur J Endocrinol **165**(6): 841-849.
He, X. S., N. Ma, Z. L. Pan, Z. X. Wang, N. Li, X. C. Zhang, J. N. Zhou, D. F. Zhu and D. R. Zhang (2011). "Functional magnetic resource imaging assessment of altered brain function in hypothyroidism during working memory processing." Eur J Endocrinol **164**(6): 951-959.
He, Y. H., X. Lu, L. Q. Yang, L. Y. Xu and Q. P. Kong (2014). "Association of the insulin-like growth factor binding protein 3 (IGFBP-3) polymorphism with longevity in Chinese nonagenarians and centenarians." Aging (Albany NY) **6**(11): 944-956.
Heaney, J. L., A. C. Phillips and D. Carroll (2012). "Ageing, physical function, and the diurnal rhythms of cortisol and dehydroepiandrosterone." Psychoneuroendocrinology **37**(3): 341-349.

Hedinger, C., E. D. Williams and L. H. Sobin (1989). "The WHO histological classification of thyroid tumors: a commentary on the second edition." Cancer **63**(5): 908-911.
Hegedus, L., H. Perrild, L. R. Poulsen, J. R. Andersen, B. Holm, P. Schnohr, G. Jensen and J. M. Hansen (1983). "The determination of thyroid volume by ultrasound and its relationship to body weight, age, and sex in normal subjects." J Clin Endocrinol Metab **56**(2): 260-263.
Hekimi, S., J. Lapointe and Y. Wen (2011). "Taking a "good" look at free radicals in the aging process." Trends Cell Biol **21**(10): 569-576.
Hendrix, A. O., C. L. Hughes and J. F. Selgrade (2014). "Modeling endocrine control of the pituitary-ovarian axis: androgenic influence and chaotic dynamics." Bull Math Biol **76**(1): 136-156.
Het, S., G. Ramlow and O. T. Wolf (2005). "A meta-analytic review of the effects of acute cortisol administration on human memory." Psychoneuroendocrinology **30**(8): 771-784.
Heuer, H. and T. J. Visser (2009). "Minireview: Pathophysiological importance of thyroid hormone transporters." Endocrinology **150**(3): 1078-1083.
Hidalgo, V., M. Almela, M. M. Pulopulos and A. Salvador (2016). "Memory performance is related to the cortisol awakening response in older people, but not to the diurnal cortisol slope." Psychoneuroendocrinology **71**: 136-146.
Hiemstra, T., K. Lim, R. Thadhani and J. E. Manson (2019). "Vitamin D and Atherosclerotic Cardiovascular Disease." J Clin Endocrinol Metab.
High, W. M., Jr., M. Briones-Galang, J. A. Clark, C. Gilkison, K. A. Mossberg, D. J. Zgaljardic, B. E. Masel and R. J. Urban (2010). "Effect of growth hormone replacement therapy on cognition after traumatic brain injury." J Neurotrauma **27**(9): 1565-1575.
Hilger, J., A. Friedel, R. Herr, T. Rausch, F. Roos, D. A. Wahl, D. D. Pierroz, P. Weber and K. Hoffmann (2014). "A systematic review of vitamin D status in populations worldwide." Br J Nutr **111**(1): 23-45.
Hill, M. N. and B. S. McEwen (2010). "Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids." Prog Neuropsychopharmacol Biol Psychiatry **34**(5): 791-797.
Hinkelmann, K., K. Wingenfeld, L. K. Kuehl, J. Fleischer, I. Heuser, K. Wiedemann and C. Otte (2015). "Stimulation of the mineralocorticoid receptor improves memory in young and elderly healthy individuals." Neurobiol Aging **36**(2): 919-924.
Hintze, G., J. Windeler, J. Baumert, H. Stein and J. Kobberling (1991). "Thyroid volume and goitre prevalence in the elderly as determined by ultrasound and their relationships to laboratory indices." Acta Endocrinol (Copenh) **124**(1): 12-18.
Hinz, B. and R. Hirschelmann (2000). "Rapid non-genomic feedback effects of glucocorticoids on CRF-induced ACTH secretion in rats." Pharm Res **17**(10): 1273-1277.
Hitt, R., Y. Young-Xu, M. Silver and T. Perls (1999). "Centenarians: the older you get, the healthier you have been." Lancet **354**(9179): 652.
Hoeijmakers, J. H. (2009). "DNA damage, aging, and cancer." N Engl J Med **361**(15): 1475-1485.
Hoenicke, L. and L. Zender (2012). "Immune surveillance of senescent cells–biological significance in cancer- and non-cancer pathologies." Carcinogenesis **33**(6): 1123-1126.
Hogervorst, E., F. Huppert, F. E. Matthews and C. Brayne (2008). "Thyroid function and cognitive decline in the MRC Cognitive Function and Ageing Study." Psychoneuroendocrinology **33**(7): 1013-1022.
Holdaway, I. M., M. J. Bolland and G. D. Gamble (2008). "A meta-analysis of the effect of lowering serum levels of GH and IGF-I on mortality in acromegaly." Eur J Endocrinol **159**(2): 89-95.
Holick, M. F. (2009). "Vitamin D status: measurement, interpretation, and clinical application." Ann Epidemiol **19**(2): 73-78.

Holick, M. F., N. C. Binkley, H. A. Bischoff-Ferrari, C. M. Gordon, D. A. Hanley, R. P. Heaney, M. H. Murad, C. M. Weaver and S. Endocrine (2011). "Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline." J Clin Endocrinol Metab **96**(7): 1911-1930.
Hollowell, J. G., N. W. Staehling, W. D. Flanders, W. H. Hannon, E. W. Gunter, C. A. Spencer and L. E. Braverman (2002). "Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III)." J Clin Endocrinol Metab **87**(2): 489-499.
Holly, J. M. and C. M. Perks (2012). "Insulin-like growth factor physiology: what we have learned from human studies." Endocrinol Metab Clin North Am **41**(2): 249-263, v.
Holzenberger, M., J. Dupont, B. Ducos, P. Leneuve, A. Geloen, P. C. Even, P. Cervera and Y. Le Bouc (2003). "IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice." Nature **421**(6919): 182-187.
Hoogendoorn, E. H., A. R. Hermus, F. de Vegt, H. A. Ross, A. L. Verbeek, L. A. Kiemeney, D. W. Swinkels, F. C. Sweep and M. den Heijer (2006). "Thyroid function and prevalence of anti-thyroperoxidase antibodies in a population with borderline sufficient iodine intake: influences of age and sex." Clin Chem **52**(1): 104-111.
Hooshmand, B., J. Lokk, A. Solomon, F. Mangialasche, J. Miralbell, G. Spulber, S. Annerbo, N. Andreasen, B. Winblad, A. Cedazo-Minguez, L. O. Wahlund and M. Kivipelto (2014). "Vitamin D in relation to cognitive impairment, cerebrospinal fluid biomarkers, and brain volumes." J Gerontol A Biol Sci Med Sci **69**(9): 1132-1138.
Horn, J. L. and R. B. Cattell (1966). "Refinement and test of the theory of fluid and crystallized general intelligences." J Educ Psychol **57**(5): 253-270.
Horn, J. L. and R. B. Cattell (1967). "Age differences in fluid and crystallized intelligence." Acta Psychol (Amst) **26**(2): 107-129.
Hornsby, P. J. (1995). "Biosynthesis of DHEAS by the human adrenal cortex and its age-related decline." Ann N Y Acad Sci **774**: 29-46.
Houston, D. K., M. Cesari, L. Ferrucci, A. Cherubini, D. Maggio, B. Bartali, M. A. Johnson, G. G. Schwartz and S. B. Kritchevsky (2007). "Association between vitamin D status and physical performance: the InCHIANTI study." J Gerontol A Biol Sci Med Sci **62**(4): 440-446.
Hu, X., Y. Yang and D. Gong (2016). "Circulating insulin-like growth factor 1 and insulin-like growth factor binding protein-3 level in Alzheimer's disease: a meta-analysis." Neurol Sci **37**(10): 1671-1677.
Hutchinson, J. (1886). "Congenital Absence of Hair and Mammary Glands with Atrophic Condition of the Skin and its Appendages, in a Boy whose Mother had been almost wholly Bald from Alopecia Areata from the age of Six." Med Chir Trans **69**: 473-477.
INE (2017). INE death statistics database. Lisbon, Instituto Nacional de Estatística.
INRJ (2007). [Portuguese Food Compositon Database]. Centro de Segurança Alimentar e Nutrição. Lisboa, Instituto Nacional Dr. Ricardo Jorge, Ministério da Saúde.
IOM (2015). Cognitive aging: Progress in under- standing and opportunities for action. Washington, DC, The National Academies Press.
Iranmanesh, A., G. Lizarralde and J. D. Veldhuis (1991). "Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men." J Clin Endocrinol Metab **73**(5): 1081-1088.
Ittermann, T., K. Wittfeld, M. Nauck, R. Bulow, N. Hosten, H. Volzke and H. J. Grabe (2018). "High Thyrotropin Is Associated with Reduced Hippocampal Volume in a Population-Based Study from Germany." Thyroid.

Jaeggi, S. M., M. Buschkuehl, J. Jonides and W. J. Perrig (2008). "Improving fluid intelligence with training on working memory." Proc Natl Acad Sci U S A **105**(19): 6829-6833.
Jaeschke, R., G. Guyatt, H. Gerstein, C. Patterson, W. Molloy, D. Cook, S. Harper, L. Griffith and R. Carbotte (1996). "Does treatment with L-thyroxine influence health status in middle-aged and older adults with subclinical hypothyroidism?" J Gen Intern Med **11**(12): 744-749.
Jagger, C., C. Gillies, F. Moscone, E. Cambois, H. Van Oyen, W. Nusselder, J. M. Robine and E. team (2008). "Inequalities in healthy life years in the 25 countries of the European Union in 2005: a cross-national meta-regression analysis." Lancet **372**(9656): 2124-2131.
Jahreis, G., W. Hausmann, G. Kiessling, K. Franke and M. Leiterer (2001). "Bioavailability of iodine from normal diets rich in dairy products–results of balance studies in women." Exp Clin Endocrinol Diabetes **109**(3): 163-167.
Jamali, N., C. M. Sorenson and N. Sheibani (2018). "Vitamin D and regulation of vascular cell function." Am J Physiol Heart Circ Physiol **314**(4): H753-H765.
Jang, W., H. J. Kim, H. Li, K. D. Jo, M. K. Lee, S. H. Song and H. O. Yang (2014). "1,25-Dyhydroxyvitamin D(3) attenuates rotenone-induced neurotoxicity in SH-SY5Y cells through induction of autophagy." Biochem Biophys Res Commun **451**(1): 142-147.
Jansen, T. A., T. I. M. Korevaar, T. A. Mulder, T. White, R. L. Muetzel, R. P. Peeters and H. Tiemeier (2019). "Maternal thyroid function during pregnancy and child brain morphology: a time window-specific analysis of a prospective cohort." Lancet Diabetes Endocrinol.
Jaracz, J., A. Kucharska, A. Rajewska-Rager and K. Lacka (2012). "Cognitive functions and mood during chronic thyrotropin-suppressive therapy with L-thyroxine in patients with differentiated thyroid carcinoma." J Endocrinol Invest **35**(8): 760-765.
Jasim, S. and H. Gharib (2018). "Thyroid and Aging." Endocr Pract **24**(4): 369-374.
Joels, M. (2006). "Corticosteroid effects in the brain: U-shape it." Trends Pharmacol Sci **27**(5): 244-250.
Jones, O. R., A. Scheuerlein, R. Salguero-Gomez, C. G. Camarda, R. Schaible, B. B. Casper, J. P. Dahlgren, J. Ehrlen, M. B. Garcia, E. S. Menges, P. F. Quintana-Ascencio, H. Caswell, A. Baudisch and J. W. Vaupel (2014). "Diversity of ageing across the tree of life." Nature **505**(7482): 169-173.
Jorde, R., K. Waterloo, H. Storhaug, A. Nyrnes, J. Sundsfjord and T. G. Jenssen (2006). "Neuropsychological function and symptoms in subjects with subclinical hypothyroidism and the effect of thyroxine treatment." J Clin Endocrinol Metab **91**(1): 145-153.
Joseph D'Ercole, A. and P. Ye (2008). "Expanding the mind: insulin-like growth factor I and brain development." Endocrinology **149**(12): 5958-5962.
Juanes, M., G. Guercio, R. Marino, E. Berensztein, D. M. Warman, M. Ciaccio, S. Gil, M. Bailez, M. A. Rivarola and A. Belgorosky (2015). "Three novel IGF1R mutations in microcephalic patients with prenatal and postnatal growth impairment." Clin Endocrinol (Oxf) **82**(5): 704-711.
Junnila, R. K., E. O. List, D. E. Berryman, J. W. Murrey and J. J. Kopchick (2013). "The GH/IGF-1 axis in ageing and longevity." Nat Rev Endocrinol **9**(6): 366-376.
Juul, A., K. Main, W. F. Blum, J. Lindholm, M. B. Ranke and N. E. Skakkebaek (1994). "The ratio between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients." Clin Endocrinol (Oxf) **41**(1): 85-93.
Kahapola-Arachchige, K. M., N. Hadlow, R. Wardrop, E. M. Lim and J. P. Walsh (2012). "Age-specific TSH reference ranges have minimal impact on the diagnosis of thyroid dysfunction." Clin Endocrinol (Oxf) **77**(5): 773-779.
Kaichi, Y., M. Kenjo, T. Higaki, F. Tatsugami, Y. Akiyama, Y. Nagata and K. Awai (2015). "Cerebral blood flow in transient hypothyroidism after thyroidectomy: Arterial spin labeling magnetic resonance study." Neuro Endocrinol Lett **36**(6): 545-551.

Kalachea, A. and I. Kickbusch (1997). "A global strategy for healthy ageing." World Health(4): 4-5.
Kalafatakis, K., G. M. Russell, C. J. Harmer, M. R. Munafo, N. Marchant, A. Wilson, J. C. Brooks, C. Durant, J. Thakrar, P. Murphy, N. J. Thai and S. L. Lightman (2018). "Ultradian rhythmicity of plasma cortisol is necessary for normal emotional and cognitive responses in man." Proc Natl Acad Sci U S A **115**(17): E4091-E4100.
Kalmijn, S., J. A. Janssen, H. A. Pols, S. W. Lamberts and M. M. Breteler (2000). "A prospective study on circulating insulin-like growth factor I (IGF-I), IGF-binding proteins, and cognitive function in the elderly." J Clin Endocrinol Metab **85**(12): 4551-4555.
Kalmijn, S., K. M. Mehta, H. A. Pols, A. Hofman, H. A. Drexhage and M. M. Breteler (2000). "Subclinical hyperthyroidism and the risk of dementia. The Rotterdam study." Clin Endocrinol (Oxf) **53**(6): 733-737.
Kalogeraki, S. (2005). Greek Mythology. Athens, Mediterraneo Editions.
Kanaley, J. A., J. Y. Weltman, K. S. Pieper, A. Weltman and M. L. Hartman (2001). "Cortisol and growth hormone responses to exercise at different times of day." J Clin Endocrinol Metab **86**(6): 2881-2889.
Kanfi, Y., S. Naiman, G. Amir, V. Peshti, G. Zinman, L. Nahum, Z. Bar-Joseph and H. Y. Cohen (2012). "The sirtuin SIRT6 regulates lifespan in male mice." Nature **483**(7388): 218-221.
Karlamangla, A. S., B. H. Singer, J. Chodosh, B. S. McEwen and T. E. Seeman (2005). "Urinary cortisol excretion as a predictor of incident cognitive impairment." Neurobiol Aging **26 Suppl 1**: 80-84.
Katznelson, L., E. R. Laws, Jr., S. Melmed, M. E. Molitch, M. H. Murad, A. Utz, J. A. Wass and S. Endocrine (2014). "Acromegaly: an endocrine society clinical practice guideline." J Clin Endocrinol Metab **99**(11): 3933-3951.
Kauppila, T. E. S., J. H. K. Kauppila and N. G. Larsson (2017). "Mammalian Mitochondria and Aging: An Update." Cell Metab **25**(1): 57-71.
Keehan, S. P., D. A. Stone, J. A. Poisal, G. A. Cuckler, A. M. Sisko, S. D. Smith, A. J. Madison, C. J. Wolfe and J. M. Lizonitz (2017). "National Health Expenditure Projections, 2016-25: Price Increases, Aging Push Sector To 20 Percent Of Economy." Health Aff (Millwood) **36**(3): 553-563.
Kent, S. T., E. K. Kabagambe, V. G. Wadley, V. J. Howard, W. L. Crosson, M. Z. Al-Hamdan, S. E. Judd, F. Peace and L. A. McClure (2014). "The relationship between long-term sunlight radiation and cognitive decline in the REGARDS cohort study." Int J Biometeorol **58**(3): 361-370.
Kern, W., C. Dodt, J. Born and H. L. Fehm (1996). "Changes in cortisol and growth hormone secretion during nocturnal sleep in the course of aging." J Gerontol A Biol Sci Med Sci **51**(1): M3-9.
Kesby, J. P., X. Cui, J. O'Loan, J. J. McGrath, T. H. Burne and D. W. Eyles (2010). "Developmental vitamin D deficiency alters dopamine-mediated behaviors and dopamine transporter function in adult female rats." Psychopharmacology (Berl) **208**(1): 159-168.
Kharchenko, V. P. (2010). Ultrasound diagnostics of thyroid diseases. Heidelberg ; New York, Springer.
Kim, J. M., R. Stewart, S. Y. Kim, K. Y. Bae, S. J. Yang, S. W. Kim, I. S. Shin and J. S. Yoon (2010). "Thyroid stimulating hormone, cognitive impairment and depression in an older korean population." Psychiatry Investig **7**(4): 264-269.
Kinney, B. A., C. J. Meliska, R. W. Steger and A. Bartke (2001). "Evidence that Ames dwarf mice age differently from their normal siblings in behavioral and learning and memory parameters." Horm Behav **39**(4): 277-284.
Kirkwood, T. B. (1977). "Evolution of ageing." Nature **270**(5635): 301-304.

Konig, F., M. Andersson, K. Hotz, I. Aeberli and M. B. Zimmermann (2011). "Ten repeat collections for urinary iodine from spot samples or 24-hour samples are needed to reliably estimate individual iodine status in women." J Nutr **141**(11): 2049-2054.
Koning, A., J. C. Buurstede, L. van Weert and O. C. Meijer (2019). "Glucocorticoid and Mineralocorticoid Receptors in the Brain: A Transcriptional Perspective." J Endocr Soc **3**(10): 1917-1930.
Kraan, G. P., R. P. Dullaart, J. J. Pratt, B. G. Wolthers, N. M. Drayer and R. De Bruin (1998). "The daily cortisol production reinvestigated in healthy men. The serum and urinary cortisol production rates are not significantly different." J Clin Endocrinol Metab **83**(4): 1247-1252.
Kuilman, T., C. Michaloglou, W. J. Mooi and D. S. Peeper (2010). "The essence of senescence." Genes Dev **24**(22): 2463-2479.
Kujoth, G. C., A. Hiona, T. D. Pugh, S. Someya, K. Panzer, S. E. Wohlgemuth, T. Hofer, A. Y. Seo, R. Sullivan, W. A. Jobling, J. D. Morrow, H. Van Remmen, J. M. Sedivy, T. Yamasoba, M. Tanokura, R. Weindruch, C. Leeuwenburgh and T. A. Prolla (2005). "Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging." Science **309**(5733): 481-484.
Kumar, G. T., H. S. Sachdev, H. Chellani, A. M. Rehman, V. Singh, H. Arora and S. Filteau (2011). "Effect of weekly vitamin D supplements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomised controlled trial." BMJ **342**: d2975.
Kumar, M., P. Rana, S. Modi, R. Tyagi, P. Kaur, R. Kanwar, T. Sekhri, M. D'Souza and S. Khushu (2019). "Aberrant intra and inter network resting state functional connectivity in thyrotoxicosis." J Neuroendocrinol **31**(2): e12683.
Kuningas, M., S. P. Mooijaart, J. Jolles, P. E. Slagboom, R. G. Westendorp and D. van Heemst (2009). "VDR gene variants associate with cognitive function and depressive symptoms in old age." Neurobiol Aging **30**(3): 466-473.
Kuzma, E., M. Soni, T. J. Littlejohns, J. M. Ranson, N. M. van Schoor, D. J. Deeg, H. Comijs, P. H. Chaves, B. R. Kestenbaum, L. H. Kuller, O. L. Lopez, J. T. Becker, K. M. Langa, W. E. Henley, I. A. Lang, O. C. Ukoumunne and D. J. Llewellyn (2016). "Vitamin D and Memory Decline: Two Population-Based Prospective Studies." J Alzheimers Dis **50**(4): 1099-1108.
LaFranchi, S. H. and S. A. Huang (2016). Hypothyroidism. Nelson Textbook of Pediatrics, Elsevier, Inc.**:** 2665-2675.
Lagouge, M., C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeq, J. Milne, P. Lambert, P. Elliott, B. Geny, M. Laakso, P. Puigserver and J. Auwerx (2006). "Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha." Cell **127**(6): 1109-1122.
Lai, Z., P. Roos, O. Zhai, Y. Olsson, K. Fholenhag, C. Larsson and F. Nyberg (1993). "Age-related reduction of human growth hormone-binding sites in the human brain." Brain Res **621**(2): 260-266.
Lam, V., M. A. Albrecht, R. Takechi, P. Prasopsang, Y. P. Lee, J. K. Foster and J. C. Mamo (2016). "Serum 25-hydroxyvitamin D is associated with reduced verbal episodic memory in healthy, middle-aged and older adults." Eur J Nutr **55**(4): 1503-1513.
Laron, Z., R. Kauli, L. Lapkina and H. Werner (2017). "IGF-I deficiency, longevity and cancer protection of patients with Laron syndrome." Mutat Res Rev Mutat Res **772**: 123-133.
Latrofa, F., E. Fiore, T. Rago, L. Antonangeli, L. Montanelli, D. Ricci, M. A. Provenzale, M. Scutari, M. Frigeri, M. Tonacchera and P. Vitti (2013). "Iodine contributes to thyroid autoimmunity in humans by unmasking a cryptic epitope on thyroglobulin." J Clin Endocrinol Metab **98**(11): E1768-1774.

Lavasani, M., A. R. Robinson, A. Lu, M. Song, J. M. Feduska, B. Ahani, J. S. Tilstra, C. H. Feldman, P. D. Robbins, L. J. Niedernhofer and J. Huard (2012). "Muscle-derived stem/progenitor cell dysfunction limits healthspan and lifespan in a murine progeria model." Nat Commun **3**: 608.
Lee, B. K., T. A. Glass, M. J. McAtee, G. S. Wand, K. Bandeen-Roche, K. I. Bolla and B. S. Schwartz (2007). "Associations of salivary cortisol with cognitive function in the Baltimore memory study." Arch Gen Psychiatry **64**(7): 810-818.
Lee, H. C. and Y. H. Wei (2012). "Mitochondria and aging." Adv Exp Med Biol **942**: 311-327.
Lee, J. S., W. O. Ward, H. Ren, B. Vallanat, G. J. Darlington, E. S. Han, J. C. Laguna, J. H. DeFord, J. Papaconstantinou, C. Selman and J. C. Corton (2012). "Meta-analysis of gene expression in the mouse liver reveals biomarkers associated with inflammation increased early during aging." Mech Ageing Dev **133**(7): 467-478.
Lefebvre d'Hellencourt, C., C. N. Montero-Menei, R. Bernard and D. Couez (2003). "Vitamin D3 inhibits proinflammatory cytokines and nitric oxide production by the EOC13 microglial cell line." J Neurosci Res **71**(4): 575-582.
Leggio, M. G., M. C. Silveri, L. Petrosini and M. Molinari (2000). "Phonological grouping is specifically affected in cerebellar patients: a verbal fluency study." J Neurol Neurosurg Psychiatry **69**(1): 102-106.
Lehmann, B. and M. Meurer (2010). "Vitamin D metabolism." Dermatol Ther **23**(1): 2-12.
Lemkine, G. F., A. Raj, G. Alfama, N. Turque, Z. Hassani, O. Alegria-Prevot, J. Samarut, G. Levi and B. A. Demeneix (2005). "Adult neural stem cell cycling in vivo requires thyroid hormone and its alpha receptor." FASEB J **19**(7): 863-865.
Lesak, M., D. Howieson, E. Bigler and D. Tranel (2012). Neuropsychological assessment. New York, Oxford university press.
Lester, E., R. K. Skinner and M. R. Wills (1977). "Seasonal variation in serum-25-hydroxyvitamin-D in the elderly in Britain." Lancet **1**(8019): 979-980.
Li, L., M. Wu, Y. Liao, L. Ouyang, M. Du, D. Lei, L. Chen, L. Yao, X. Huang and Q. Gong (2014). "Grey matter reduction associated with posttraumatic stress disorder and traumatic stress." Neurosci Biobehav Rev **43**: 163-172.
Li, L., M. Zhi, Z. Hou, Y. Zhang, Y. Yue and Y. Yuan (2017). "Abnormal brain functional connectivity leads to impaired mood and cognition in hyperthyroidism: a resting-state functional MRI study." Oncotarget **8**(4): 6283-6294.
Licht, C. M., L. C. van Turenhout, J. B. Deijen, L. L. Koppes, W. van Mechelen, J. W. Twisk and M. L. Drent (2014). "The Association between IGF-1 Polymorphisms, IGF-1 Serum Levels, and Cognitive Functions in Healthy Adults: The Amsterdam Growth and Health Longitudinal Study." Int J Endocrinol **2014**: 181327.
Lichtenwalner, R. J., M. E. Forbes, S. A. Bennett, C. D. Lynch, W. E. Sonntag and D. R. Riddle (2001). "Intracerebroventricular infusion of insulin-like growth factor-I ameliorates the age-related decline in hippocampal neurogenesis." Neuroscience **107**(4): 603-613.
Lievre, A., F. Jusot, T. Barnay, C. Sermet, N. Brouard, J. M. Robine, M. A. Brieu and F. Forette (2007). "Healthy working life expectancies at age 50 in Europe: a new indicator." J Nutr Health Aging **11**(6): 508-514.
Lim, A. S. P., C. Gaiteri, L. Yu, S. Sohail, W. Swardfager, S. Tasaki, J. A. Schneider, C. Paquet, D. T. Stuss, M. Masellis, S. E. Black, J. Hugon, A. S. Buchman, L. L. Barnes, D. A. Bennett and P. L. De Jager (2018). "Seasonal plasticity of cognition and related biological measures in adults with and without Alzheimer disease: Analysis of multiple cohorts." PLoS Med **15**(9): e1002647.
Limbert, E., S. Prazeres, M. Sao Pedro, D. Madureira, A. Miranda, M. Ribeiro, F. Carrilho, J. Jacome de Castro, M. S. Lopes, J. Cardoso, A. Carvalho, M. J. Oliveira, H. Reguengo, F. Borges and M.

Grupo de Estudos da Tiroide da Sociedade Portuguesa de Endocrinologia Diabetes e (2012). "[Iodine intake in Portuguese school children]." Acta Med Port **25**(1): 29-36.
Lin, S., L. W. Fan, P. G. Rhodes and Z. Cai (2009). "Intranasal administration of IGF-1 attenuates hypoxic-ischemic brain injury in neonatal rats." Exp Neurol **217**(2): 361-370.
Lin, S. J., M. Kaeberlein, A. A. Andalis, L. A. Sturtz, P. A. Defossez, V. C. Culotta, G. R. Fink and L. Guarente (2002). "Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration." Nature **418**(6895): 344-348.
Lipsitz, L. A. and A. L. Goldberger (1992). "Loss of 'complexity' and aging. Potential applications of fractals and chaos theory to senescence." JAMA **267**(13): 1806-1809.
List, E. O., D. E. Berryman, K. Funk, E. S. Gosney, A. Jara, B. Kelder, X. Wang, L. Kutz, K. Troike, N. Lozier, V. Mikula, E. R. Lubbers, H. Zhang, C. Vesel, R. K. Junnila, S. J. Frank, M. M. Masternak, A. Bartke and J. J. Kopchick (2013). "The role of GH in adipose tissue: lessons from adipose-specific GH receptor gene-disrupted mice." Mol Endocrinol **27**(3): 524-535.
Liston, C. and W. B. Gan (2011). "Glucocorticoids are critical regulators of dendritic spine development and plasticity in vivo." Proc Natl Acad Sci U S A **108**(38): 16074-16079.
Liu, B., Q. Ran, D. Liu, S. Zhang and D. Zhang (2017). "Changes in Resting-State Cerebral Activity in Patients with Hyperthyroidism: A Short-Term Follow-Up Functional MR Imaging Study." Sci Rep **7**(1): 10627.
Liu, H., D. M. Bravata, I. Olkin, S. Nayak, B. Roberts, A. M. Garber and A. R. Hoffman (2007). "Systematic review: the safety and efficacy of growth hormone in the healthy elderly." Ann Intern Med **146**(2): 104-115.
Liu, N., M. Landreh, K. Cao, M. Abe, G. J. Hendriks, J. R. Kennerdell, Y. Zhu, L. S. Wang and N. M. Bonini (2012). "The microRNA miR-34 modulates ageing and neurodegeneration in Drosophila." Nature **482**(7386): 519-523.
Liu, X., Z. Bai, F. Liu, M. Li, Q. Zhang, G. Song and J. Xu (2012). "Reduced posterior cingulate glutamate measured by magnetic resonance spectroscopy in hyperthyroidism." Neuro Endocrinol Lett **33**(6): 626-630.
Livner, A., A. Wahlin and L. Backman (2009). "Thyroid stimulating hormone and prospective memory functioning in old age." Psychoneuroendocrinology **34**(10): 1554-1559.
Liyanarachchi, K., R. Ross and M. Debono (2017). "Human studies on hypothalamo-pituitary-adrenal (HPA) axis." Best Pract Res Clin Endocrinol Metab **31**(5): 459-473.
Llewellyn, D. J., I. A. Lang, K. M. Langa and D. Melzer (2011). "Vitamin D and cognitive impairment in the elderly U.S. population." J Gerontol A Biol Sci Med Sci **66**(1): 59-65.
Llewellyn, D. J., I. A. Lang, K. M. Langa, G. Muniz-Terrera, C. L. Phillips, A. Cherubini, L. Ferrucci and D. Melzer (2010). "Vitamin D and risk of cognitive decline in elderly persons." Arch Intern Med **170**(13): 1135-1141.
Logan, S., G. A. Pharaoh, M. C. Marlin, D. R. Masser, S. Matsuzaki, B. Wronowski, A. Yeganeh, E. E. Parks, P. Premkumar, J. A. Farley, D. B. Owen, K. M. Humphries, M. Kinter, W. M. Freeman, L. I. Szweda, H. Van Remmen and W. E. Sonntag (2018). "Insulin-like growth factor receptor signaling regulates working memory, mitochondrial metabolism, and amyloid-beta uptake in astrocytes." Mol Metab **9**: 141-155.
Lopez-Otin, C., M. A. Blasco, L. Partridge, M. Serrano and G. Kroemer (2013). "The hallmarks of aging." Cell **153**(6): 1194-1217.
Lorenz, E. N. (1963). "Deterministic non-periodic flow." Journal of the Atmospheric Sciences **20**(2): 130–141.
Lucassen, P. J., J. Pruessner, N. Sousa, O. F. Almeida, A. M. Van Dam, G. Rajkowska, D. F. Swaab and B. Czeh (2014). "Neuropathology of stress." Acta Neuropathol **127**(1): 109-135.

Lupien, S. J., M. de Leon, S. de Santi, A. Convit, C. Tarshish, N. P. Nair, M. Thakur, B. S. McEwen, R. L. Hauger and M. J. Meaney (1998). "Cortisol levels during human aging predict hippocampal atrophy and memory deficits." Nat Neurosci **1**(1): 69-73.
Mackey, M. C. and L. Glass (1977). "Oscillation and chaos in physiological control systems." Science **197**(4300): 287-289.
MacLaughlin, J. and M. F. Holick (1985). "Aging decreases the capacity of human skin to produce vitamin D3." J Clin Invest **76**(4): 1536-1538.
MacLullich, A. M., I. J. Deary, J. M. Starr, K. J. Ferguson, J. M. Wardlaw and J. R. Seckl (2005). "Plasma cortisol levels, brain volumes and cognition in healthy elderly men." Psychoneuroendocrinology **30**(5): 505-515.
Madeira, M. D., N. Sousa, M. T. Lima-Andrade, F. Calheiros, A. Cadete-Leite and M. M. Paula-Barbosa (1992). "Selective vulnerability of the hippocampal pyramidal neurons to hypothyroidism in male and female rats." J Comp Neurol **322**(4): 501-518.
Maegawa, S., G. Hinkal, H. S. Kim, L. Shen, L. Zhang, J. Zhang, N. Zhang, S. Liang, L. A. Donehower and J. P. Issa (2010). "Widespread and tissue specific age-related DNA methylation changes in mice." Genome Res **20**(3): 332-340.
Magarinos, A. M. and B. S. McEwen (1995). "Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors." Neuroscience **69**(1): 89-98.
Maggio, M., A. Ble, G. P. Ceda and E. J. Metter (2006). "Decline in insulin-like growth factor-I levels across adult life span in two large population studies." J Gerontol A Biol Sci Med Sci **61**(2): 182-183.
Magri, F., B. Muzzoni, L. Cravello, M. Fioravanti, L. Busconi, D. Camozzi, G. Vignati and E. Ferrari (2002). "Thyroid function in physiological aging and in centenarians: possible relationships with some nutritional markers." Metabolism **51**(1): 105-109.
Mahmoud, G. S. and L. M. Grover (2006). "Growth hormone enhances excitatory synaptic transmission in area CA1 of rat hippocampus." J Neurophysiol **95**(5): 2962-2974.
Mahne, A., G. El-Haddad, A. Alavi, M. Houseni, G. Moonis, A. Mong, M. Hernandez-Pampaloni and D. A. Torigian (2007). "Assessment of age-related morphological and functional changes of selected structures of the head and neck by computed tomography, magnetic resonance imaging, and positron emission tomography." Semin Nucl Med **37**(2): 88-102.
Makeham, W. M. (1860). "On the Law of Mortality and the Construction of Annuity Tables." J. Inst. Actuaries and Assur. Mag.(8): 301-310.
Mammen, J. S., J. McGready, P. W. Ladenson and E. M. Simonsick (2017). "Unstable Thyroid Function in Older Adults Is Caused by Alterations in Both Thyroid and Pituitary Physiology and Is Associated with Increased Mortality." Thyroid **27**(11): 1370-1377.
Man, E. B. and W. S. Jones (1969). "Thyroid function in human pregnancy. V. Incidence of maternal serum low butanol-extractable iodines and of normal gestational TBG and TBPA capacities; retardation of 8-month-old infants." Am J Obstet Gynecol **104**(6): 898-908.
Manard, M., D. Carabin, M. Jaspar and F. Collette (2014). "Age-related decline in cognitive control: the role of fluid intelligence and processing speed." BMC Neurosci **15**: 7.
Maravall, F. J., N. Gomez-Arnaiz, A. Guma, R. Abos, J. Soler and J. M. Gomez (2004). "Reference values of thyroid volume in a healthy, non-iodine-deficient Spanish population." Horm Metab Res **36**(9): 645-649.
Maril, A., P. E. Davis, J. J. Koo, N. Reggev, M. Zuckerman, L. Ehrenfeld, R. V. Mulkern, D. P. Waber and M. J. Rivkin (2010). "Developmental fMRI study of episodic verbal memory encoding in children." Neurology **75**(23): 2110-2116.

Mariotti, S., G. Barbesino, P. Caturegli, L. Bartalena, P. Sansoni, F. Fagnoni, D. Monti, U. Fagiolo, C. Franceschi and A. Pinchera (1993). "Complex alteration of thyroid function in healthy centenarians." J Clin Endocrinol Metab **77**(5): 1130-1134.
Mariotti, S., P. Sansoni, G. Barbesino, P. Caturegli, D. Monti, A. Cossarizza, T. Giacomelli, G. Passeri, U. Fagiolo, A. Pinchera and et al. (1992). "Thyroid and other organ-specific autoantibodies in healthy centenarians." Lancet **339**(8808): 1506-1508.
Markowska, A. L., M. Mooney and W. E. Sonntag (1998). "Insulin-like growth factor-1 ameliorates age-related behavioral deficits." Neuroscience **87**(3): 559-569.
Marques, P. C., J. M. Soares, R. J. Magalhaes, N. C. Santos and N. J. Sousa (2016). "Macro- and micro-structural white matter differences correlate with cognitive performance in healthy aging." Brain Imaging Behav **10**(1): 168-181.
Marsili, A., A. M. Zavacki, J. W. Harney and P. R. Larsen (2011). "Physiological role and regulation of iodothyronine deiodinases: a 2011 update." J Endocrinol Invest **34**(5): 395-407.
Martin, J. and C. Li (2016). Normal Cognitive Aging. Brocklehurst's Textbook of Geriatric Medicine and Gerontology. Howard Fillit, Kenneth Rockwood and J. B. Young, Elsevier Inc. **:** 170-177.
Masoro, E. J. (2016). The Physiology of Aging. Medical Physiology. Walter Boron and E. Boulpaep, Elsevier**:** 1235-1247.
Matheu, A., A. Maraver, M. Collado, I. Garcia-Cao, M. Canamero, C. Borras, J. M. Flores, P. Klatt, J. Vina and M. Serrano (2009). "Anti-aging activity of the Ink4/Arf locus." Aging Cell **8**(2): 152-161.
Matthews, B. R. (2015). "Memory dysfunction." Continuum (Minneap Minn) **21**(3 Behavioral Neurology and Neuropsychiatry): 613-626.
Matusz, P. J., H. Broadbent, J. Ferrari, B. Forrest, R. Merkley and G. Scerif (2015). "Multi-modal distraction: insights from children's limited attention." Cognition **136**: 156-165.
Mayerl, S., J. Muller, R. Bauer, S. Richert, C. M. Kassmann, V. M. Darras, K. Buder, A. Boelen, T. J. Visser and H. Heuer (2014). "Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis." J Clin Invest **124**(5): 1987-1999.
McEwen, B. S. (1998). "Protective and damaging effects of stress mediators." N Engl J Med **338**(3): 171-179.
McEwen, B. S., N. P. Bowles, J. D. Gray, M. N. Hill, R. G. Hunter, I. N. Karatsoreos and C. Nasca (2015). "Mechanisms of stress in the brain." Nat Neurosci **18**(10): 1353-1363.
McEwen, B. S. and J. H. Morrison (2013). "The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course." Neuron **79**(1): 16-29.
McEwen, B. S., C. Nasca and J. D. Gray (2016). "Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex." Neuropsychopharmacology **41**(1): 3-23.
McEwen, B. S., J. M. Weiss and L. S. Schwartz (1968). "Selective retention of corticosterone by limbic structures in rat brain." Nature **220**(5170): 911-912.
McMorris, F. A., T. M. Smith, S. DeSalvo and R. W. Furlanetto (1986). "Insulin-like growth factor I/somatomedin C: a potent inducer of oligodendrocyte development." Proc Natl Acad Sci U S A **83**(3): 822-826.
Medawar, P. B. (1946). "Old age and natural death." Mod. Q.(2): 30-49.
Medici, M., W. E. Visser, T. J. Visser and R. P. Peeters (2015). "Genetic determination of the hypothalamic-pituitary-thyroid axis: where do we stand?" Endocr Rev **36**(2): 214-244.
Medvedev, Z. A. (1990). "An attempt at a rational classification of theories of ageing." Biol Rev Camb Philos Soc **65**(3): 375-398.
Meier-Ruge, W., J. Ulrich, M. Bruhlmann and E. Meier (1992). "Age-related white matter atrophy in the human brain." Ann N Y Acad Sci **673**: 260-269.

Melmed, S. (2017). The pituitary. London, United Kingdom ; San Diego, CA, Elsevier/Academic Press.
Mendes-de-Aguiar, C. B., R. Alchini, H. Decker, M. Alvarez-Silva, C. I. Tasca and A. G. Trentin (2008). "Thyroid hormone increases astrocytic glutamate uptake and protects astrocytes and neurons against glutamate toxicity." J Neurosci Res **86**(14): 3117-3125.
Mendoza, A. and A. N. Hollenberg (2017). "New insights into thyroid hormone action." Pharmacol Ther **173**: 135-145.
Mesquita, A. R., Y. Wegerich, A. V. Patchev, M. Oliveira, P. Leao, N. Sousa and O. F. Almeida (2009). "Glucocorticoids and neuro- and behavioural development." Semin Fetal Neonatal Med **14**(3): 130-135.
Meyer, C., V. Muto, M. Jaspar, C. Kusse, E. Lambot, S. L. Chellappa, C. Degueldre, E. Balteau, A. Luxen, B. Middleton, S. N. Archer, F. Collette, D. J. Dijk, C. Phillips, P. Maquet and G. Vandewalle (2016). "Seasonality in human cognitive brain responses." Proc Natl Acad Sci U S A **113**(11): 3066-3071.
Miao, Q., S. Zhang, Y. H. Guan, H. Y. Ye, Z. Y. Zhang, Q. Y. Zhang, R. D. Xue, M. F. Zeng, C. T. Zuo and Y. M. Li (2011). "Reversible changes in brain glucose metabolism following thyroid function normalization in hyperthyroidism." AJNR Am J Neuroradiol **32**(6): 1034-1042.
Michos, E. D., K. A. Carson, A. L. Schneider, P. L. Lutsey, L. Xing, A. R. Sharrett, A. Alonso, L. H. Coker, M. Gross, W. Post, T. H. Mosley and R. F. Gottesman (2014). "Vitamin D and subclinical cerebrovascular disease: the Atherosclerosis Risk in Communities brain magnetic resonance imaging study." JAMA Neurol **71**(7): 863-871.
Mick, P., D. M. Foley and F. R. Lin (2014). "Hearing loss is associated with poorer ratings of patient-physician communication and healthcare quality." J Am Geriatr Soc **62**(11): 2207-2209.
Midford, R. and K. Kirsner (2005). "Implicit and Explicit Learning in Aged and Young Adults." Neuropsychol Dev Cogn B Aging Neuropsychol Cogn **12**(4): 359-387.
Milman, S., G. Atzmon, D. M. Huffman, J. Wan, J. P. Crandall, P. Cohen and N. Barzilai (2014). "Low insulin-like growth factor-1 level predicts survival in humans with exceptional longevity." Aging Cell **13**(4): 769-771.
Mion, M., K. Patterson, J. Acosta-Cabronero, G. Pengas, D. Izquierdo-Garcia, Y. T. Hong, T. D. Fryer, G. B. Williams, J. R. Hodges and P. J. Nestor (2010). "What the left and right anterior fusiform gyri tell us about semantic memory." Brain **133**(11): 3256-3268.
Mitchell, D. B. (1989). "How many memory systems? Evidence from aging." J Exp Psychol Learn Mem Cogn **15**(1): 31-49.
Mizoguchi, K., T. Kunishita, D. H. Chui and T. Tabira (1992). "Stress induces neuronal death in the hippocampus of castrated rats." Neurosci Lett **138**(1): 157-160.
Molina, P. E. (2018). Endocrine Physiology, 5th edition, McGraw-Hill Education.
Molinari, C., F. Uberti, E. Grossini, G. Vacca, S. Carda, M. Invernizzi and C. Cisari (2011). "1alpha,25-dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells." Cell Physiol Biochem **27**(6): 661-668.
Monchi, O., M. Petrides, A. P. Strafella, K. J. Worsley and J. Doyon (2006). "Functional role of the basal ganglia in the planning and execution of actions." Ann Neurol **59**(2): 257-264.
Monzani, F., P. Del Guerra, N. Caraccio, C. A. Pruneti, E. Pucci, M. Luisi and L. Baschieri (1993). "Subclinical hypothyroidism: neurobehavioral features and beneficial effect of L-thyroxine treatment." Clin Investig **71**(5): 367-371.
Moog, N. K., S. Entringer, C. Heim, P. D. Wadhwa, N. Kathmann and C. Buss (2017). "Influence of maternal thyroid hormones during gestation on fetal brain development." Neuroscience **342**: 68-100.

Moon, J. H., Y. J. Park, T. H. Kim, J. W. Han, S. H. Choi, S. Lim, D. J. Park, K. W. Kim and H. C. Jang (2014). "Lower-but-normal serum TSH level is associated with the development or progression of cognitive impairment in elderly: Korean Longitudinal Study on Health and Aging (KLoSHA)." J Clin Endocrinol Metab **99**(2): 424-432.
Morano, M. I., D. M. Vazquez and H. Akil (1994). "The role of the hippocampal mineralocorticoid and glucocorticoid receptors in the hypothalamo-pituitary-adrenal axis of the aged Fisher rat." Mol Cell Neurosci **5**(5): 400-412.
Moreau, O. K., C. Cortet-Rudelli, E. Yollin, E. Merlen, W. Daveluy and M. Rousseaux (2013). "Growth hormone replacement therapy in patients with traumatic brain injury." J Neurotrauma **30**(11): 998-1006.
Moreira, P. S., N. Santos, T. Castanho, L. Amorim, C. Portugal-Nunes, N. Sousa and P. Costa (2018). "Longitudinal measurement invariance of memory performance and executive functioning in healthy aging." PLoS One **13**(9): e0204012.
Morreale de Escobar, G., M. J. Obregon and F. Escobar del Rey (2000). "Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia?" J Clin Endocrinol Metab **85**(11): 3975-3987.
Morrison, J. H. and M. G. Baxter (2012). "The ageing cortical synapse: hallmarks and implications for cognitive decline." Nat Rev Neurosci **13**(4): 240-250.
Moskalev, A. A., M. V. Shaposhnikov, E. N. Plyusnina, A. Zhavoronkov, A. Budovsky, H. Yanai and V. E. Fraifeld (2013). "The role of DNA damage and repair in aging through the prism of Koch-like criteria." Ageing Res Rev **12**(2): 661-684.
Muller, E. E., S. G. Cella, V. De Gennaro Colonna, M. Parenti, D. Cocchi and V. Locatelli (1993). "Aspects of the neuroendocrine control of growth hormone secretion in ageing mammals." J Reprod Fertil Suppl **46**: 99-114.
Muller, M. B., P. J. Lucassen, A. Yassouridis, W. J. Hoogendijk, F. Holsboer and D. F. Swaab (2001). "Neither major depression nor glucocorticoid treatment affects the cellular integrity of the human hippocampus." Eur J Neurosci **14**(10): 1603-1612.
Muller, R. A. (2017). Now: The Physics of Time, W. W. Norton & Company.
Nashiro, K., J. Guevara-Aguirre, M. N. Braskie, G. W. Hafzalla, R. Velasco, P. Balasubramanian, M. Wei, P. M. Thompson, M. Mather, M. D. Nelson, A. Guevara, E. Teran and V. D. Longo (2017). "Brain Structure and Function Associated with Younger Adults in Growth Hormone Receptor-Deficient Humans." J Neurosci **37**(7): 1696-1707.
Nath, S. K., B. Moinier, F. Thuillier, M. Rongier and J. F. Desjeux (1992). "Urinary excretion of iodide and fluoride from supplemented food grade salt." Int J Vitam Nutr Res **62**(1): 66-72.
Neff, F., D. Flores-Dominguez, D. P. Ryan, M. Horsch, S. Schroder, T. Adler, L. C. Afonso, J. A. Aguilar-Pimentel, L. Becker, L. Garrett, W. Hans, M. M. Hettich, R. Holtmeier, S. M. Holter, K. Moreth, C. Prehn, O. Puk, I. Racz, B. Rathkolb, J. Rozman, B. Naton, R. Ordemann, J. Adamski, J. Beckers, R. Bekeredjian, D. H. Busch, G. Ehninger, J. Graw, H. Hofler, M. Klingenspor, T. Klopstock, M. Ollert, J. Stypmann, E. Wolf, W. Wurst, A. Zimmer, H. Fuchs, V. Gailus-Durner, M. Hrabe de Angelis and D. Ehninger (2013). "Rapamycin extends murine lifespan but has limited effects on aging." J Clin Invest **123**(8): 3272-3291.
Nelson, G., J. Wordsworth, C. Wang, D. Jurk, C. Lawless, C. Martin-Ruiz and T. von Zglinicki (2012). "A senescent cell bystander effect: senescence-induced senescence." Aging Cell **11**(2): 345-349.
Newcomer, J. W., G. Selke, A. K. Melson, T. Hershey, S. Craft, K. Richards and A. L. Alderson (1999). "Decreased memory performance in healthy humans induced by stress-level cortisol treatment." Arch Gen Psychiatry **56**(6): 527-533.

Ng, L., A. Hernandez, W. He, T. Ren, M. Srinivas, M. Ma, V. A. Galton, D. L. St Germain and D. Forrest (2009). "A protective role for type 3 deiodinase, a thyroid hormone-inactivating enzyme, in cochlear development and auditory function." Endocrinology **150**(4): 1952-1960.
Nicholson, J. L. and J. Altman (1972). "The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. I. Cell proliferation and differentiation." Brain Res **44**(1): 13-23.
Nicholson, J. L. and J. Altman (1972). "Synaptogenesis in the rat cerebellum: effects of early hypo- and hyperthyroidism." Science **176**(4034): 530-532.
Noguchi, T. (1996). "Effects of growth hormone on cerebral development: morphological studies." Horm Res **45**(1-2): 5-17.
Nunez, A., E. Carro and I. Torres-Aleman (2003). "Insulin-like growth factor I modifies electrophysiological properties of rat brain stem neurons." J Neurophysiol **89**(6): 3008-3017.
Nyakas, C., I. Granic, L. G. Halmy, P. Banerjee and P. G. Luiten (2011). "The basal forebrain cholinergic system in aging and dementia. Rescuing cholinergic neurons from neurotoxic amyloid-beta42 with memantine." Behav Brain Res **221**(2): 594-603.
Nyberg, F. (2000). "Growth hormone in the brain: characteristics of specific brain targets for the hormone and their functional significance." Front Neuroendocrinol **21**(4): 330-348.
Nyberg, F. and P. Burman (1996). "Growth hormone and its receptors in the central nervous system–location and functional significance." Horm Res **45**(1-2): 18-22.
Nyberg, F. and M. Hallberg (2013). "Growth hormone and cognitive function." Nat Rev Endocrinol **9**(6): 357-365.
Nye, E. J., J. E. Grice, G. I. Hockings, C. R. Strakosch, G. V. Crosbie, M. M. Walters, D. J. Torpy and R. V. Jackson (2001). "The insulin hypoglycemia test: hypoglycemic criteria and reproducibility." J Neuroendocrinol **13**(6): 524-530.
O'Brien, J. T., I. Schweitzer, D. Ames, V. Tuckwell and M. Mastwyk (1994). "Cortisol suppression by dexamethasone in the healthy elderly: effects of age, dexamethasone levels, and cognitive function." Biol Psychiatry **36**(6): 389-394.
O'Brien, M. D. and P. W. Harris (1968). "Cerebral-cortex perfusion-rates in myxoedema." Lancet **1**(7553): 1170-1172.
O'Hanlon, J. F., J. J. McGrath and M. E. McCauley (1974). "Body temperature and temporal acuity." J Exp Psychol **102**(5): 788-794.
Oberdoerffer, P. and D. A. Sinclair (2007). "The role of nuclear architecture in genomic instability and ageing." Nat Rev Mol Cell Biol **8**(9): 692-702.
OECD (2015). Ageing: Debate the Issues, OECD Insights. Paris, OECD Publishing.
Oetting, A. and P. M. Yen (2007). "New insights into thyroid hormone action." Best Pract Res Clin Endocrinol Metab **21**(2): 193-208.
Ohlsson, C., S. Mohan, K. Sjogren, A. Tivesten, J. Isgaard, O. Isaksson, J. O. Jansson and J. Svensson (2009). "The role of liver-derived insulin-like growth factor-I." Endocr Rev **30**(5): 494-535.
Okereke, O., J. H. Kang, J. Ma, S. E. Hankinson, M. N. Pollak and F. Grodstein (2007). "Plasma IGF-I levels and cognitive performance in older women." Neurobiol Aging **28**(1): 135-142.
Okereke, O. I., J. H. Kang, J. Ma, J. M. Gaziano and F. Grodstein (2006). "Midlife plasma insulin-like growth factor I and cognitive function in older men." J Clin Endocrinol Metab **91**(11): 4306-4312.
Olsen, G. G. and A. V. Everitt (1965). "Retardation of the ageing process in collagen fibres from the tail tendon of the old hypophysectomized rat." Nature **206**(981): 307-308.

Onken, B. and M. Driscoll (2010). "Metformin induces a dietary restriction-like state and the oxidative stress response to extend C. elegans Healthspan via AMPK, LKB1, and SKN-1." PLoS One **5**(1): e8758.
Ord, W. M. (1888). "Report of a committee of the Clinical Society of London nominated to investigate the subject of myxoedema." Trans Clin Soc Lond(Supp 21).
Orgel, L. E. (1963). "The maintenance of the accuracy of protein synthesis and its relevance to ageing." Proc Natl Acad Sci U S A **49**: 517-521.
Osterweil, D., K. Syndulko, S. N. Cohen, P. D. Pettler-Jennings, J. M. Hershman, J. L. Cummings, W. W. Tourtellotte and D. H. Solomon (1992). "Cognitive function in non-demented older adults with hypothyroidism." J Am Geriatr Soc **40**(4): 325-335.
Ostrowski, P. P., A. Barszczyk, J. Forstenpointner, W. Zheng and Z. P. Feng (2016). "Meta-Analysis of Serum Insulin-Like Growth Factor 1 in Alzheimer's Disease." PLoS One **11**(5): e0155733.
Otte, C., K. Wingenfeld, L. K. Kuehl, S. Richter, F. Regen, D. Piber and K. Hinkelmann (2015). "Cognitive function in older adults with major depression: Effects of mineralocorticoid receptor stimulation." J Psychiatr Res **69**: 120-125.
Over, R., S. Mannan, H. Nsouli-Maktabi, K. D. Burman and J. Jonklaas (2010). "Age and the thyrotropin response to hypothyroxinemia." J Clin Endocrinol Metab **95**(8): 3675-3683.
Overbergh, L., B. Decallonne, D. Valckx, A. Verstuyf, J. Depovere, J. Laureys, O. Rutgeerts, R. Saint-Arnaud, R. Bouillon and C. Mathieu (2000). "Identification and immune regulation of 25-hydroxyvitamin D-1-alpha-hydroxylase in murine macrophages." Clin Exp Immunol **120**(1): 139-146.
Oxford, D. (2011). Time, Oxford University Press.
Palacios, N., T. Scott, N. Sahasrabudhe, X. Gao and K. L. Tucker (2019). "Serum vitamin D and cognition in a cohort of Boston-area Puerto Ricans." Nutr Neurosci: 1-8.
Panici, J. A., J. M. Harper, R. A. Miller, A. Bartke, A. Spong and M. M. Masternak (2010). "Early life growth hormone treatment shortens longevity and decreases cellular stress resistance in long-lived mutant mice." FASEB J **24**(12): 5073-5079.
Papadakis, M. A., D. Grady, D. Black, M. J. Tierney, G. A. Gooding, M. Schambelan and C. Grunfeld (1996). "Growth hormone replacement in healthy older men improves body composition but not functional ability." Ann Intern Med **124**(8): 708-716.
Papadimitriou, D. T. (2017). "The Big Vitamin D Mistake." J Prev Med Public Health **50**(4): 278-281.
Pardo, J., M. C. Abba, E. Lacunza, O. M. Ogundele, I. Paiva, G. R. Morel, T. F. Outeiro and R. G. Goya (2018). "IGF-I Gene Therapy in Aging Rats Modulates Hippocampal Genes Relevant to Memory Function." J Gerontol A Biol Sci Med Sci **73**(4): 459-467.
Pardo, J., M. Uriarte, G. M. Console, P. C. Reggiani, T. F. Outeiro, G. R. Morel and R. G. Goya (2016). "Insulin-like growth factor-I gene therapy increases hippocampal neurogenesis, astrocyte branching and improves spatial memory in female aging rats." Eur J Neurosci **44**(4): 2120-2128.
Park, D. C. and A. H. Gutchess (2002). "Aging, cognition, and culture: a neuroscientific perspective." Neurosci Biobehav Rev **26**(7): 859-867.
Parkin, A. J. and A. Lawrence (1994). "A dissociation in the relation between memory tasks and frontal lobe tests in the normal elderly." Neuropsychologia **32**(12): 1523-1532.
Parle, J., L. Roberts, S. Wilson, H. Pattison, A. Roalfe, M. S. Haque, C. Heath, M. Sheppard, J. Franklyn and F. D. Hobbs (2010). "A randomized controlled trial of the effect of thyroxine replacement on cognitive function in community-living elderly subjects with subclinical hypothyroidism: the Birmingham Elderly Thyroid study." J Clin Endocrinol Metab **95**(8): 3623-3632.

Parle, J. V., J. A. Franklyn, K. W. Cross, S. C. Jones and M. C. Sheppard (1991). "Prevalence and follow-up of abnormal thyrotrophin (TSH) concentrations in the elderly in the United Kingdom." Clin Endocrinol (Oxf) **34**(1): 77-83.
Parreira, D., M. C. Serra and M. G. Dias (2015). "[Vitamin D levels in products dairy products consumed in Portugal]." Boletim Epidemiologico do Instituto Nacional Dr. Ricardo Jorge(12): 27-28.
Parsaik, A. K., B. Singh, R. O. Roberts, S. Pankratz, K. K. Edwards, Y. E. Geda, H. Gharib, B. F. Boeve, D. S. Knopman and R. C. Petersen (2014). "Hypothyroidism and risk of mild cognitive impairment in elderly persons: a population-based study." JAMA Neurol **71**(2): 201-207.
Paschke, R. (2011). "Molecular pathogenesis of nodular goiter." Langenbecks Arch Surg **396**(8): 1127-1136.
Pasqualetti, G., G. Pagano, G. Rengo, N. Ferrara and F. Monzani (2015). "Subclinical Hypothyroidism and Cognitive Impairment: Systematic Review and Meta-Analysis." J Clin Endocrinol Metab **100**(11): 4240-4248.
Patel, R., J. Williams-Dautovich and C. L. Cummins (2014). "Minireview: new molecular mediators of glucocorticoid receptor activity in metabolic tissues." Mol Endocrinol **28**(7): 999-1011.
Paulo, A. C., A. Sampaio, N. C. Santos, P. S. Costa, P. Cunha, J. Zihl, J. Cerqueira, J. A. Palha and N. Sousa (2011). "Patterns of cognitive performance in healthy ageing in Northern Portugal: a cross-sectional analysis." PLoS One **6**(9): e24553.
Paulsen, A. J., C. R. Schubert, A. Pinto, C. M. Carlsson, R. J. Chappell, M. E. Fischer, B. E. K. Klein, R. Klein, M. Y. Tsai and K. J. Cruickshanks (2019). "Neuroprotective Biomarkers and Cognitive Function in a Long-Term Prospective Population-based Study of Aging US Adults." Alzheimer Dis Assoc Disord.
Pavlides, C., Y. Watanabe, A. M. Magarinos and B. S. McEwen (1995). "Opposing roles of type I and type II adrenal steroid receptors in hippocampal long-term potentiation." Neuroscience **68**(2): 387-394.
Pawlikowska, L., D. Hu, S. Huntsman, A. Sung, C. Chu, J. Chen, A. H. Joyner, N. J. Schork, W. C. Hsueh, A. P. Reiner, B. M. Psaty, G. Atzmon, N. Barzilai, S. R. Cummings, W. S. Browner, P. Y. Kwok, E. Ziv and F. Study of Osteoporotic (2009). "Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity." Aging Cell **8**(4): 460-472.
Pereira, F., A. Barbachano, P. K. Singh, M. J. Campbell, A. Munoz and M. J. Larriba (2012). "Vitamin D has wide regulatory effects on histone demethylase genes." Cell Cycle **11**(6): 1081-1089.
Perez, V. I., H. Van Remmen, A. Bokov, C. J. Epstein, J. Vijg and A. Richardson (2009). "The overexpression of major antioxidant enzymes does not extend the lifespan of mice." Aging Cell **8**(1): 73-75.
Perice, L., N. Barzilai, J. Verghese, E. F. Weiss, R. Holtzer, P. Cohen and S. Milman (2016). "Lower circulating insulin-like growth factor-I is associated with better cognition in females with exceptional longevity without compromise to muscle mass and function." Aging (Albany NY) **8**(10): 2414-2424.
Perkiomaki, J. S., T. H. Makikallio and H. V. Huikuri (2005). "Fractal and complexity measures of heart rate variability." Clin Exp Hypertens **27**(2-3): 149-158.
Perna, L., U. Mons, M. Kliegel and H. Brenner (2014). "Serum 25-hydroxyvitamin D and cognitive decline: a longitudinal study among non-demented older adults." Dement Geriatr Cogn Disord **38**(3-4): 254-263.
Perretti, M., A. Ahluwalia, J. G. Harris, H. J. Harris, S. K. Wheller and R. J. Flower (1996). "Acute inflammatory response in the mouse: exacerbation by immunoneutralization of lipocortin 1." Br J Pharmacol **117**(6): 1145-1154.

Pettersen, J. A. (2016). "Vitamin D and executive functioning: Are higher levels better?" J Clin Exp Neuropsychol **38**(4): 467-477.
Pettersen, J. A. (2017). "Does high dose vitamin D supplementation enhance cognition?: A randomized trial in healthy adults." Exp Gerontol **90**: 90-97.
Pharoah, P. O., I. H. Buttfield and B. S. Hetzel (1971). "Neurological damage to the fetus resulting from severe iodine deficiency during pregnancy." Lancet **1**(7694): 308-310.
Pharoah, P. O., K. J. Connolly, R. P. Ekins and A. G. Harding (1984). "Maternal thyroid hormone levels in pregnancy and the subsequent cognitive and motor performance of the children." Clin Endocrinol (Oxf) **21**(3): 265-270.
Pilecka, I., A. Whatmore, R. Hooft van Huijsduijnen, B. Destenaves and P. Clayton (2007). "Growth hormone signalling: sprouting links between pathways, human genetics and therapeutic options." Trends Endocrinol Metab **18**(1): 12-18.
Pilling, L. C., L. W. Harries, J. Powell, D. J. Llewellyn, L. Ferrucci and D. Melzer (2012). "Genomics and successful aging: grounds for renewed optimism?" J Gerontol A Biol Sci Med Sci **67**(5): 511-519.
Pilo, A., G. Iervasi, F. Vitek, M. Ferdeghini, F. Cazzuola and R. Bianchi (1990). "Thyroidal and peripheral production of 3,5,3'-triiodothyronine in humans by multicompartmental analysis." Am J Physiol **258**(4 Pt 1): E715-726.
Pilz, S., A. Zittermann, C. Trummer, V. Theiler-Schwetz, E. Lerchbaum, M. H. Keppel, M. R. Grubler, W. Marz and M. Pandis (2019). "Vitamin D testing and treatment: a narrative review of current evidence." Endocr Connect **8**(2): R27-R43.
Pinchera, A., S. Mariotti, G. Barbesino, R. Bechi, P. Sansoni, U. Fagiolo, A. Cossarizza and C. Franceschi (1995). "Thyroid autoimmunity and ageing." Horm Res **43**(1-3): 64-68.
Pino, S., S. L. Fang and L. E. Braverman (1998). "Ammonium persulfate: a new and safe method for measuring urinary iodine by ammonium persulfate oxidation." Exp Clin Endocrinol Diabetes **106 Suppl 3**: S22-27.
Pludowski, P., M. F. Holick, S. Pilz, C. L. Wagner, B. W. Hollis, W. B. Grant, Y. Shoenfeld, E. Lerchbaum, D. J. Llewellyn, K. Kienreich and M. Soni (2013). "Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-a review of recent evidence." Autoimmun Rev **12**(10): 976-989.
Poincaré, H. (1890). "Sur le problème des trois corps et les équations de la dynamique." Acta mathematica(13): 1-270.
Pollock, M. A., A. Sturrock, K. Marshall, K. M. Davidson, C. J. Kelly, A. D. McMahon and E. H. McLaren (2001). "Thyroxine treatment in patients with symptoms of hypothyroidism but thyroid function tests within the reference range: randomised double blind placebo controlled crossover trial." BMJ **323**(7318): 891-895.
Pop, V. J., J. L. Kuijpens, A. L. van Baar, G. Verkerk, M. M. van Son, J. J. de Vijlder, T. Vulsma, W. M. Wiersinga, H. A. Drexhage and H. L. Vader (1999). "Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy." Clin Endocrinol (Oxf) **50**(2): 149-155.
PORDATA (2018). PORDATA database. Lisbon, Francisco Manuel dos Santos Foundation.
Porterfield, S. P. and C. E. Hendrich (1993). "The role of thyroid hormones in prenatal and neonatal neurological development–current perspectives." Endocr Rev **14**(1): 94-106.
Potvin, O., H. Forget, M. Preville, D. Berbiche, Y. C. Chagnon and C. Hudon (2013). "Relationship between cortisol level and prevalent/incident cognitive impairment and its moderating factors in older adults." Int Psychogeriatr **25**(2): 252-262.

Powe, C. E., M. K. Evans, J. Wenger, A. B. Zonderman, A. H. Berg, M. Nalls, H. Tamez, D. Zhang, I. Bhan, S. A. Karumanchi, N. R. Powe and R. Thadhani (2013). "Vitamin D-binding protein and vitamin D status of black Americans and white Americans." N Engl J Med **369**(21): 1991-2000.
Powers, E. T., R. I. Morimoto, A. Dillin, J. W. Kelly and W. E. Balch (2009). "Biological and chemical approaches to diseases of proteostasis deficiency." Annu Rev Biochem **78**: 959-991.
Premack, D. (2007). "Human and animal cognition: continuity and discontinuity." Proc Natl Acad Sci U S A **104**(35): 13861-13867.
Preston, J. D., L. J. Reynolds and K. J. Pearson (2018). "Developmental Origins of Health Span and Life Span: A Mini-Review." Gerontology **64**(3): 237-245.
Prezioso, G., C. Giannini and F. Chiarelli (2018). "Effect of Thyroid Hormones on Neurons and Neurodevelopment." Horm Res Paediatr **90**(2): 73-81.
Prinz, P. N., J. M. Scanlan, P. P. Vitaliano, K. E. Moe, S. Borson, B. Toivola, G. R. Merriam, L. H. Larsen and H. L. Reed (1999). "Thyroid hormones: positive relationships with cognition in healthy, euthyroid older men." J Gerontol A Biol Sci Med Sci **54**(3): M111-116.
Prufer, K., T. D. Veenstra, G. F. Jirikowski and R. Kumar (1999). "Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the rat brain and spinal cord." J Chem Neuroanat **16**(2): 135-145.
Przybelski, R., S. Agrawal, D. Krueger, J. A. Engelke, F. Walbrun and N. Binkley (2008). "Rapid correction of low vitamin D status in nursing home residents." Osteoporos Int **19**(11): 1621-1628.
Puig, K. L., J. A. Kulas, W. Franklin, S. G. Rakoczy, G. Taglialatela, H. M. Brown-Borg and C. K. Combs (2016). "The Ames dwarf mutation attenuates Alzheimer's disease phenotype of APP/PS1 mice." Neurobiol Aging **40**: 22-40.
Pulopulos, M. M., V. Hidalgo, M. Almela, S. Puig-Perez, C. Villada and A. Salvador (2014). "Hair cortisol and cognitive performance in healthy older people." Psychoneuroendocrinology **44**: 100-111.
Purnell, J. Q., D. D. Brandon, L. M. Isabelle, D. L. Loriaux and M. H. Samuels (2004). "Association of 24-hour cortisol production rates, cortisol-binding globulin, and plasma-free cortisol levels with body composition, leptin levels, and aging in adult men and women." J Clin Endocrinol Metab **89**(1): 281-287.
Pyka, G., R. A. Wiswell and R. Marcus (1992). "Age-dependent effect of resistance exercise on growth hormone secretion in people." J Clin Endocrinol Metab **75**(2): 404-407.
Quann, E. E., V. L. Fulgoni, 3rd and N. Auestad (2015). "Consuming the daily recommended amounts of dairy products would reduce the prevalence of inadequate micronutrient intakes in the United States: diet modeling study based on NHANES 2007-2010." Nutr J **14**: 90.
Quax, R. A., L. Manenschijn, J. W. Koper, J. M. Hazes, S. W. Lamberts, E. F. van Rossum and R. A. Feelders (2013). "Glucocorticoid sensitivity in health and disease." Nat Rev Endocrinol **9**(11): 670-686.
Quinque, E. M., S. Karger, K. Arelin, M. L. Schroeter, J. Kratzsch and A. Villringer (2014). "Structural and functional MRI study of the brain, cognition and mood in long-term adequately treated Hashimoto's thyroiditis." Psychoneuroendocrinology **42**: 188-198.
Rabinovici, G. D., M. L. Stephens and K. L. Possin (2015). "Executive dysfunction." Continuum (Minneap Minn) **21**(3 Behavioral Neurology and Neuropsychiatry): 646-659.
Ragnarsson, O., A. Stomby, P. Dahlqvist, J. A. Evang, M. Ryberg, T. Olsson, J. Bollerslev, L. Nyberg and G. Johannsson (2017). "Decreased prefrontal functional brain response during memory testing in women with Cushing's syndrome in remission." Psychoneuroendocrinology **82**: 117-125.
Rajaram, S., D. J. Baylink and S. Mohan (1997). "Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions." Endocr Rev **18**(6): 801-831.

Ramagopalan, S. V., A. Heger, A. J. Berlanga, N. J. Maugeri, M. R. Lincoln, A. Burrell, L. Handunnetthi, A. E. Handel, G. Disanto, S. M. Orton, C. T. Watson, J. M. Morahan, G. Giovannoni, C. P. Ponting, G. C. Ebers and J. C. Knight (2010). "A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution." Genome Res **20**(10): 1352-1360.

Ramos, H. E. (2014). "Thyroid hormone cell membrane transport defect." Endocr Dev **26**: 108-117.

Ramscar, M., P. Hendrix, C. Shaoul, P. Milin and H. Baayen (2014). "The myth of cognitive decline: non-linear dynamics of lifelong learning." Top Cogn Sci **6**(1): 5-42.

Rando, T. A. and H. Y. Chang (2012). "Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock." Cell **148**(1-2): 46-57.

Rando, T. A. and T. Wyss-Coray (2014). "Stem cells as vehicles for youthful regeneration of aged tissues." J Gerontol A Biol Sci Med Sci **69 Suppl 1**: S39-42.

Raposo, L., S. Martins, D. Ferreira, J. T. Guimaraes and A. C. Santos (2017). "Vitamin D, parathyroid hormone and metabolic syndrome - the PORMETS study." BMC Endocr Disord **17**(1): 71.

Rapp, P. R. and J. Bachevalier (2012). Cognitive Development and Aging. Fundamental Neuroscience. E. Inc.

Ravaglia, G., P. Forti, F. Maioli, F. Boschi, M. Bernardi, L. Pratelli, A. Pizzoferrato and G. Gasbarrini (1996). "The relationship of dehydroepiandrosterone sulfate (DHEAS) to endocrine-metabolic parameters and functional status in the oldest-old. Results from an Italian study on healthy free-living over-ninety-year-olds." J Clin Endocrinol Metab **81**(3): 1173-1178.

Raz, N., U. Lindenberger, K. M. Rodrigue, K. M. Kennedy, D. Head, A. Williamson, C. Dahle, D. Gerstorf and J. D. Acker (2005). "Regional brain changes in aging healthy adults: general trends, individual differences and modifiers." Cereb Cortex **15**(11): 1676-1689.

Reagan, L. P. and B. S. McEwen (1997). "Controversies surrounding glucocorticoid-mediated cell death in the hippocampus." J Chem Neuroanat **13**(3): 149-167.

Resta, F., V. Triggiani, G. Barile, M. Benigno, P. Suppressa, V. A. Giagulli, E. Guastamacchia and C. Sabba (2012). "Subclinical hypothyroidism and cognitive dysfunction in the elderly." Endocr Metab Immune Disord Drug Targets **12**(3): 260-267.

Reul, J. M. and E. R. de Kloet (1985). "Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation." Endocrinology **117**(6): 2505-2511.

Richards, J. B., A. M. Valdes, J. P. Gardner, D. Paximadas, M. Kimura, A. Nessa, X. Lu, G. L. Surdulescu, R. Swaminathan, T. D. Spector and A. Aviv (2007). "Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women." Am J Clin Nutr **86**(5): 1420-1425.

Ridgway, E. C., B. D. Weintraub and F. Maloof (1974). "Metabolic clearance and production rates of human thyrotropin." J Clin Invest **53**(3): 895-903.

Rieben, C., D. Segna, B. R. da Costa, T. H. Collet, L. Chaker, C. E. Aubert, C. Baumgartner, O. P. Almeida, E. Hogervorst, S. Trompet, K. Masaki, S. P. Mooijaart, J. Gussekloo, R. P. Peeters, D. C. Bauer, D. Aujesky and N. Rodondi (2016). "Subclinical Thyroid Dysfunction and the Risk of Cognitive Decline: a Meta-Analysis of Prospective Cohort Studies." J Clin Endocrinol Metab **101**(12): 4945-4954.

Rimmele, U., L. Besedovsky, T. Lange and J. Born (2013). "Blocking mineralocorticoid receptors impairs, blocking glucocorticoid receptors enhances memory retrieval in humans." Neuropsychopharmacology **38**(5): 884-894.

Ristow, M. and S. Schmeisser (2011). "Extending life span by increasing oxidative stress." Free Radic Biol Med **51**(2): 327-336.

Rizzolatti, G., L. Riggio, I. Dascola and C. Umilta (1987). "Reorienting attention across the horizontal and vertical meridians: evidence in favor of a premotor theory of attention." Neuropsychologia **25**(1A): 31-40.
Robbins, T. W. and A. F. Arnsten (2009). "The neuropsychopharmacology of fronto-executive function: monoaminergic modulation." Annu Rev Neurosci **32**: 267-287.
Roberts, L. M., H. Pattison, A. Roalfe, J. Franklyn, S. Wilson, F. D. Hobbs and J. V. Parle (2006). "Is subclinical thyroid dysfunction in the elderly associated with depression or cognitive dysfunction?" Ann Intern Med **145**(8): 573-581.
Robine, J. M. and K. Ritchie (1991). "Healthy life expectancy: evaluation of global indicator of change in population health." BMJ **302**(6774): 457-460.
Roelfsema, F., H. Pijl, D. M. Keenan and J. D. Veldhuis (2012). "Diminished adrenal sensitivity and ACTH efficacy in obese premenopausal women." Eur J Endocrinol **167**(5): 633-642.
Roelfsema, F., H. Pijl, P. Kok, E. Endert, E. Fliers, N. R. Biermasz, A. M. Pereira and J. D. Veldhuis (2014). "Thyrotropin secretion in healthy subjects is robust and independent of age and gender, and only weakly dependent on body mass index." J Clin Endocrinol Metab **99**(2): 570-578.
Roelfsema, F., D. van Heemst, A. Iranmanesh, P. Takahashi, R. Yang and J. D. Veldhuis (2017). "Impact of age, sex and body mass index on cortisol secretion in 143 healthy adults." Endocr Connect **6**(7): 500-509.
Roelfsema, F. and J. D. Veldhuis (2013). "Thyrotropin secretion patterns in health and disease." Endocr Rev **34**(5): 619-657.
Rose, M. R. (1991). Evolutionary Biology of Aging.
Rosen, C. J. (2011). "Clinical practice. Vitamin D insufficiency." N Engl J Med **364**(3): 248-254.
Rosenfalck, A. M., S. Maghsoudi, S. Fisker, J. O. Jorgensen, J. S. Christiansen, J. Hilsted, A. A. Volund and S. Madsbad (2000). "The effect of 30 months of low-dose replacement therapy with recombinant human growth hormone (rhGH) on insulin and C-peptide kinetics, insulin secretion, insulin sensitivity, glucose effectiveness, and body composition in GH-deficient adults." J Clin Endocrinol Metab **85**(11): 4173-4181.
Ross, A. C., J. E. Manson, S. A. Abrams, J. F. Aloia, P. M. Brannon, S. K. Clinton, R. A. Durazo-Arvizu, J. C. Gallagher, R. L. Gallo, G. Jones, C. S. Kovacs, S. T. Mayne, C. J. Rosen and S. A. Shapses (2011). "The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know." J Clin Endocrinol Metab **96**(1): 53-58.
Rossi, D. J., D. Bryder, J. Seita, A. Nussenzweig, J. Hoeijmakers and I. L. Weissman (2007). "Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age." Nature **447**(7145): 725-729.
Rossom, R. C., M. A. Espeland, J. E. Manson, M. W. Dysken, K. C. Johnson, D. S. Lane, E. S. LeBlanc, F. A. Lederle, K. H. Masaki and K. L. Margolis (2012). "Calcium and vitamin D supplementation and cognitive impairment in the women's health initiative." J Am Geriatr Soc **60**(12): 2197-2205.
Rovelli, C. (2018). The Order of Time, Riverhead Books.
Rowe, J. B., I. Toni, O. Josephs, R. S. Frackowiak and R. E. Passingham (2000). "The prefrontal cortex: response selection or maintenance within working memory?" Science **288**(5471): 1656-1660.
Rowe, J. W. and R. L. Kahn (1987). "Human aging: usual and successful." Science **237**(4811): 143-149.
Rowe, J. W. and R. L. Kahn (2015). "Successful Aging 2.0: Conceptual Expansions for the 21st Century." J Gerontol B Psychol Sci Soc Sci **70**(4): 593-596.
Rubinsztein, D. C., G. Marino and G. Kroemer (2011). "Autophagy and aging." Cell **146**(5): 682-695.

Rudman, D., A. G. Feller, H. S. Nagraj, G. A. Gergans, P. Y. Lalitha, A. F. Goldberg, R. A. Schlenker, L. Cohn, I. W. Rudman and D. E. Mattson (1990). "Effects of human growth hormone in men over 60 years old." N Engl J Med **323**(1): 1-6.
Ruiz-Marcos, A., F. Sanchez-Toscano, F. Escobar del Rey and G. Morreale de Escobar (1979). "Severe hypothyroidism and the maturation of the rat cerebral cortex." Brain Res **162**(2): 315-329.
Russell, E., G. Koren, M. Rieder and S. Van Uum (2012). "Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions." Psychoneuroendocrinology **37**(5): 589-601.
Russell, W., R. F. Harrison, N. Smith, K. Darzy, S. Shalet, A. P. Weetman and R. J. Ross (2008). "Free triiodothyronine has a distinct circadian rhythm that is delayed but parallels thyrotropin levels." J Clin Endocrinol Metab **93**(6): 2300-2306.
Rutjes, A. W., D. A. Denton, M. Di Nisio, L. Y. Chong, R. P. Abraham, A. S. Al-Assaf, J. L. Anderson, M. A. Malik, R. W. Vernooij, G. Martinez, N. Tabet and J. McCleery (2018). "Vitamin and mineral supplementation for maintaining cognitive function in cognitively healthy people in mid and late life." Cochrane Database Syst Rev **12**: CD011906.
Ryan, Z. C., T. A. Craig, C. D. Folmes, X. Wang, I. R. Lanza, N. S. Schaible, J. L. Salisbury, K. S. Nair, A. Terzic, G. C. Sieck and R. Kumar (2016). "1alpha,25-Dihydroxyvitamin D3 Regulates Mitochondrial Oxygen Consumption and Dynamics in Human Skeletal Muscle Cells." J Biol Chem **291**(3): 1514-1528.
Saaltink, D. J. and E. Vreugdenhil (2014). "Stress, glucocorticoid receptors, and adult neurogenesis: a balance between excitation and inhibition?" Cell Mol Life Sci **71**(13): 2499-2515.
Sabe, L., L. Jason, M. Juejati, R. Leiguarda and S. E. Starkstein (1995). "Dissociation between declarative and procedural learning in dementia and depression." J Clin Exp Neuropsychol **17**(6): 841-848.
Salat, D. H., R. L. Buckner, A. Z. Snyder, D. N. Greve, R. S. Desikan, E. Busa, J. C. Morris, A. M. Dale and B. Fischl (2004). "Thinning of the cerebral cortex in aging." Cereb Cortex **14**(7): 721-730.
Salminen, A., K. Kaarniranta and A. Kauppinen (2012). "Inflammaging: disturbed interplay between autophagy and inflammasomes." Aging (Albany NY) **4**(3): 166-175.
Salomon, F., R. C. Cuneo, R. Hesp and P. H. Sonksen (1989). "The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency." N Engl J Med **321**(26): 1797-1803.
Salthouse, T. A. and E. J. Meinz (1995). "Aging, inhibition, working memory, and speed." J Gerontol B Psychol Sci Soc Sci **50**(6): P297-306.
Salvatore, D., T. F. Davies, M.-J. Schlumberger, I. D. Hay and P. R. Larsen (2011). Thyroid Physiology and Diagnostic Evaluation of Patients with Thyroid Disorders. Williams Textbook of Endocrinology. Elsevier**:** 327–361.
Samuels, M. H., R. Kaimal, A. Waring, H. A. Fink, K. Yaffe, A. R. Hoffman, E. Orwoll and D. Bauer (2016). "Thyroid Function Variations Within the Reference Range Do Not Affect Quality of Life, Mood, or Cognitive Function in Community-Dwelling Older Men." Thyroid **26**(9): 1185-1194.
Samuels, M. H., I. Kolobova, A. Smeraglio, M. Niederhausen, J. S. Janowsky and K. G. Schuff (2016). "Effect of Thyroid Function Variations Within the Laboratory Reference Range on Health Status, Mood, and Cognition in Levothyroxine-Treated Subjects." Thyroid **26**(9): 1173-1184.
Samuels, M. H., I. Kolobova, A. Smeraglio, D. Peters, J. S. Janowsky and K. G. Schuff (2014). "The effects of levothyroxine replacement or suppressive therapy on health status, mood, and cognition." J Clin Endocrinol Metab **99**(3): 843-851.

Samuels, M. H., K. G. Schuff, N. E. Carlson, P. Carello and J. S. Janowsky (2008). "Health status, mood, and cognition in experimentally induced subclinical thyrotoxicosis." J Clin Endocrinol Metab **93**(5): 1730-1736.
Samuels, M. H., J. D. Veldhuis, P. Henry and E. C. Ridgway (1990). "Pathophysiology of pulsatile and copulsatile release of thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone, and alpha-subunit." J Clin Endocrinol Metab **71**(2): 425-432.
Sanchez, M. M., L. J. Young, P. M. Plotsky and T. R. Insel (2000). "Distribution of corticosteroid receptors in the rhesus brain: relative absence of glucocorticoid receptors in the hippocampal formation." J Neurosci **20**(12): 4657-4668.
SanMartin, C. D., M. Henriquez, C. Chacon, D. P. Ponce, F. Salech, N. K. Rogers and M. I. Behrens (2018). "Vitamin D Increases Abeta140 Plasma Levels and Protects Lymphocytes from Oxidative Death in Mild Cognitive Impairment Patients." Curr Alzheimer Res **15**(6): 561-569.
Santos, A., T. F. Amaral, R. S. Guerra, A. S. Sousa, L. Alvares, P. Moreira, P. Padrao, C. Afonso and N. Borges (2017). "Vitamin D status and associated factors among Portuguese older adults: results from the Nutrition UP 65 cross-sectional study." BMJ Open **7**(6): e016123.
Santos, N. C., P. S. Costa, P. Cunha, J. Cotter, A. Sampaio, J. Zihl, O. F. Almeida, J. J. Cerqueira, J. A. Palha and N. Sousa (2013). "Mood is a key determinant of cognitive performance in community-dwelling older adults: a cross-sectional analysis." Age (Dordr) **35**(5): 1983-1993.
Santos, N. C., P. S. Costa, P. Cunha, C. Portugal-Nunes, L. Amorim, J. Cotter, J. J. Cerqueira, J. A. Palha and N. Sousa (2014). "Clinical, physical and lifestyle variables and relationship with cognition and mood in aging: a cross-sectional analysis of distinct educational groups." Front Aging Neurosci **6**: 21.
Sapolsky, R. M. (2000). "Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders." Arch Gen Psychiatry **57**(10): 925-935.
Sapolsky, R. M., L. C. Krey and B. S. McEwen (1986). "The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis." Endocr Rev **7**(3): 284-301.
Sapolsky, R. M., H. Uno, C. S. Rebert and C. E. Finch (1990). "Hippocampal damage associated with prolonged glucocorticoid exposure in primates." J Neurosci **10**(9): 2897-2902.
Sassi, F. (2006). "Calculating QALYs, comparing QALY and DALY calculations." Health Policy Plan **21**(5): 402-408.
Sato, H., T. Takahashi, K. Sumitani, H. Takatsu and S. Urano (2010). "Glucocorticoid Generates ROS to Induce Oxidative Injury in the Hippocampus, Leading to Impairment of Cognitive Function of Rats." J Clin Biochem Nutr **47**(3): 224-232.
Schapiro, S., K. Vukovich and A. Globus (1973). "Effects of neonatal thyroxine and hydrocortisone administration on the development of dendritic spines in the visual cortex of rats." Exp Neurol **40**(2): 286-296.
Scheel, M., A. Strohle and H. Bruhn (2010). "Effects of short-term stress-like cortisol on cerebral metabolism: a proton magnetic resonance spectroscopy study at 3.0 T." J Psychiatr Res **44**(8): 521-526.
Schilbach, K. and M. Bidlingmaier (2015). "Growth hormone binding protein - physiological and analytical aspects." Best Pract Res Clin Endocrinol Metab **29**(5): 671-683.
Schmahmann, J. D. and D. N. Pandya (2008). "Disconnection syndromes of basal ganglia, thalamus, and cerebrocerebellar systems." Cortex **44**(8): 1037-1066.
Schneider, A. L. C., D. Zhao, P. L. Lutsey, R. F. Gottesman, A. R. Sharrett, A. M. Rawlings, A. Alonso, D. Knopman, T. H. Mosley, E. Selvin and E. D. Michos (2018). "Serum Vitamin D Concentrations and Cognitive Change Over 20 Years: The Atherosclerosis Risk in Communities Neurocognitive Study." Neuroepidemiology **51**(3-4): 131-137.

Schoofs, D., D. Preuss and O. T. Wolf (2008). "Psychosocial stress induces working memory impairments in an n-back paradigm." Psychoneuroendocrinology **33**(5): 643-653.
Schottker, B., R. Jorde, A. Peasey, B. Thorand, E. H. Jansen, L. Groot, M. Streppel, J. Gardiner, J. M. Ordonez-Mena, L. Perna, T. Wilsgaard, W. Rathmann, E. Feskens, E. Kampman, G. Siganos, I. Njolstad, E. B. Mathiesen, R. Kubinova, A. Pajak, R. Topor-Madry, A. Tamosiunas, M. Hughes, F. Kee, M. Bobak, A. Trichopoulou, P. Boffetta, H. Brenner, H. Consortium on, E. Ageing: Network of Cohorts in and S. the United (2014). "Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States." BMJ **348**: g3656.
Schöttker, B., R. Jorde, A. Peasey, B. Thorand, E. H. J. M. Jansen, L. d. Groot, M. Streppel, J. Gardiner, J. M. Ordóñez-Mena, L. Perna, T. Wilsgaard, W. Rathmann, E. Feskens, E. Kampman, G. Siganos, I. Njølstad, E. B. Mathiesen, R. Kubínová, A. Pająk, R. Topor-Madry, A. Tamosiunas, M. Hughes, F. Kee, M. Bobak, A. Trichopoulou, P. Boffetta and H. Brenner (2014). "Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States." BMJ **348**.
Schreckenberger, M. F., U. T. Egle, S. Drecker, H. G. Buchholz, M. M. Weber, P. Bartenstein and G. J. Kahaly (2006). "Positron emission tomography reveals correlations between brain metabolism and mood changes in hyperthyroidism." J Clin Endocrinol Metab **91**(12): 4786-4791.
Schubert, M. I., R. Kalisch, I. Sotiropoulos, C. Catania, N. Sousa, O. F. Almeida and D. P. Auer (2008). "Effects of altered corticosteroid milieu on rat hippocampal neurochemistry and structure--an in vivo magnetic resonance spectroscopy and imaging study." J Psychiatr Res **42**(11): 902-912.
Schumacher, B., I. van der Pluijm, M. J. Moorhouse, T. Kosteas, A. R. Robinson, Y. Suh, T. M. Breit, H. van Steeg, L. J. Niedernhofer, W. van Ijcken, A. Bartke, S. R. Spindler, J. H. Hoeijmakers, G. T. van der Horst and G. A. Garinis (2008). "Delayed and accelerated aging share common longevity assurance mechanisms." PLoS Genet **4**(8): e1000161.
Schurmeyer, T. H., P. C. Avgerinos, P. W. Gold, W. T. Gallucci, T. P. Tomai, G. B. Cutler, Jr., D. L. Loriaux and G. P. Chrousos (1984). "Human corticotropin-releasing factor in man: pharmacokinetic properties and dose-response of plasma adrenocorticotropin and cortisol secretion." J Clin Endocrinol Metab **59**(6): 1103-1108.
Schuster, I. (2011). "Cytochromes P450 are essential players in the vitamin D signaling system." Biochim Biophys Acta **1814**(1): 186-199.
Schwabe, L., O. Hoffken, M. Tegenthoff and O. T. Wolf (2013). "Stress-induced enhancement of response inhibition depends on mineralocorticoid receptor activation." Psychoneuroendocrinology **38**(10): 2319-2326.
Schwartz, C. E., M. M. May, N. J. Carpenter, R. C. Rogers, J. Martin, M. G. Bialer, J. Ward, J. Sanabria, S. Marsa, J. A. Lewis, R. Echeverri, H. A. Lubs, K. Voeller, R. J. Simensen and R. E. Stevenson (2005). "Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene." Am J Hum Genet **77**(1): 41-53.
Seeman, T. E., B. S. McEwen, B. H. Singer, M. S. Albert and J. W. Rowe (1997). "Increase in urinary cortisol excretion and memory declines: MacArthur studies of successful aging." J Clin Endocrinol Metab **82**(8): 2458-2465.
Seeman, T. E., B. Singer and P. Charpentier (1995). "Gender differences in patterns of HPA axis response to challenge: Macarthur studies of successful aging." Psychoneuroendocrinology **20**(7): 711-725.
Shaw, A. C., S. Joshi, H. Greenwood, A. Panda and J. M. Lord (2010). "Aging of the innate immune system." Curr Opin Immunol **22**(4): 507-513.

Sheline, Y. I., M. Sanghavi, M. A. Mintun and M. H. Gado (1999). "Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression." J Neurosci **19**(12): 5034-5043.
Sheline, Y. I., P. W. Wang, M. H. Gado, J. G. Csernansky and M. W. Vannier (1996). "Hippocampal atrophy in recurrent major depression." Proc Natl Acad Sci U S A **93**(9): 3908-3913.
Sherman, B., C. Wysham and B. Pfohl (1985). "Age-related changes in the circadian rhythm of plasma cortisol in man." J Clin Endocrinol Metab **61**(3): 439-443.
Shields, G. S., J. C. Bonner and W. G. Moons (2015). "Does cortisol influence core executive functions? A meta-analysis of acute cortisol administration effects on working memory, inhibition, and set-shifting." Psychoneuroendocrinology **58**: 91-103.
Shields, G. S., M. A. Sazma and A. P. Yonelinas (2016). "The effects of acute stress on core executive functions: A meta-analysis and comparison with cortisol." Neurosci Biobehav Rev **68**: 651-668.
Siedlecki, K. L., Y. Stern, A. Reuben, R. L. Sacco, M. S. Elkind and C. B. Wright (2009). "Construct validity of cognitive reserve in a multiethnic cohort: The Northern Manhattan Study." J Int Neuropsychol Soc **15**(4): 558-569.
Singh-Manoux, A., A. Dugravot, A. Elbaz, M. Shipley, M. Kivimaki and M. Kumari (2014). "No evidence of a longitudinal association between diurnal cortisol patterns and cognition." Neurobiol Aging **35**(10): 2239-2245.
Singh, S., M. Kumar, S. Modi, P. Kaur, L. R. Shankar and S. Khushu (2015). "Alterations of Functional Connectivity Among Resting-State Networks in Hypothyroidism." J Neuroendocrinol **27**(7): 609-615.
Slinin, Y., M. Paudel, B. C. Taylor, A. Ishani, R. Rossom, K. Yaffe, T. Blackwell, L. Y. Lui, M. Hochberg, K. E. Ensrud and G. Study of Osteoporotic Fractures Research (2012). "Association between serum 25(OH) vitamin D and the risk of cognitive decline in older women." J Gerontol A Biol Sci Med Sci **67**(10): 1092-1098.
Slinin, Y., M. L. Paudel, B. C. Taylor, H. A. Fink, A. Ishani, M. T. Canales, K. Yaffe, E. Barrett-Connor, E. S. Orwoll, J. M. Shikany, E. S. Leblanc, J. A. Cauley, K. E. Ensrud and G. Osteoporotic Fractures in Men Study Research (2010). "25-Hydroxyvitamin D levels and cognitive performance and decline in elderly men." Neurology **74**(1): 33-41.
Someya, S., W. Yu, W. C. Hallows, J. Xu, J. M. Vann, C. Leeuwenburgh, M. Tanokura, J. M. Denu and T. A. Prolla (2010). "Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction." Cell **143**(5): 802-812.
Sonntag, W. E., C. S. Carter, Y. Ikeno, K. Ekenstedt, C. S. Carlson, R. F. Loeser, S. Chakrabarty, S. Lee, C. Bennett, R. Ingram, T. Moore and M. Ramsey (2005). "Adult-onset growth hormone and insulin-like growth factor I deficiency reduces neoplastic disease, modifies age-related pathology, and increases life span." Endocrinology **146**(7): 2920-2932.
Sonntag, W. E., R. W. Steger, L. J. Forman and J. Meites (1980). "Decreased pulsatile release of growth hormone in old male rats." Endocrinology **107**(6): 1875-1879.
Souchay, C. and M. Isingrini (2004). "Age related differences in metacognitive control: role of executive functioning." Brain Cogn **56**(1): 89-99.
Sousa, N. (2016). "The dynamics of the stress neuromatrix." Mol Psychiatry **21**(3): 302-312.
Sousa, N. and O. F. Almeida (2002). "Corticosteroids: sculptors of the hippocampal formation." Rev Neurosci **13**(1): 59-84.
Sousa, N., M. M. Paula-Barbosa and O. F. Almeida (1999). "Ligand and subfield specificity of corticoid-induced neuronal loss in the rat hippocampal formation." Neuroscience **89**(4): 1079-1087.

Spaniol, J., D. J. Madden and A. Voss (2006). "A diffusion model analysis of adult age differences in episodic and semantic long-term memory retrieval." J Exp Psychol Learn Mem Cogn **32**(1): 101-117.
Speakman, J. R., C. Selman, J. S. McLaren and E. J. Harper (2002). "Living fast, dying when? The link between aging and energetics." J Nutr **132**(6 Suppl 2): 1583S-1597S.
Spencer, R. L. and T. Deak (2017). "A users guide to HPA axis research." Physiol Behav **178**: 43-65.
Spencer, W. D. and N. Raz (1995). "Differential effects of aging on memory for content and context: a meta-analysis." Psychol Aging **10**(4): 527-539.
Spiga, F., J. J. Walker, J. R. Terry and S. L. Lightman (2014). "HPA axis-rhythms." Compr Physiol **4**(3): 1273-1298.
Stan, M. and J. C. Morris (2005). "Thyrotropin-axis adaptation in aging and chronic disease." Endocrinol Metab Clin North Am **34**(4): 973-992, x.
Starkman, M. N., S. S. Gebarski, S. Berent and D. E. Schteingart (1992). "Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome." Biol Psychiatry **32**(9): 756-765.
Starkman, M. N., B. Giordani, S. S. Gebarski, S. Berent, M. A. Schork and D. E. Schteingart (1999). "Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease." Biol Psychiatry **46**(12): 1595-1602.
Stavreva, D. A., M. Wiench, S. John, B. L. Conway-Campbell, M. A. McKenna, J. R. Pooley, T. A. Johnson, T. C. Voss, S. L. Lightman and G. L. Hager (2009). "Ultradian hormone stimulation induces glucocorticoid receptor-mediated pulses of gene transcription." Nat Cell Biol **11**(9): 1093-1102.
Stawski, R. S., D. M. Almeida, M. E. Lachman, P. A. Tun, C. B. Rosnick and T. Seeman (2011). "Associations between cognitive function and naturally occurring daily cortisol during middle adulthood: timing is everything." J Gerontol B Psychol Sci Soc Sci **66 Suppl 1**: i71-81.
Stein, M. S., S. C. Scherer, K. S. Ladd and L. C. Harrison (2011). "A randomized controlled trial of high-dose vitamin D2 followed by intranasal insulin in Alzheimer's disease." J Alzheimers Dis **26**(3): 477-484.
Stern, Y. (2002). "What is cognitive reserve? Theory and research application of the reserve concept." J Int Neuropsychol Soc **8**(3): 448-460.
Stern, Y. (2009). "Cognitive reserve." Neuropsychologia **47**(10): 2015-2028.
Stern, Y. (2012). "Cognitive reserve in ageing and Alzheimer's disease." Lancet Neurol **11**(11): 1006-1012.
Steyn, F. J., V. Tolle, C. Chen and J. Epelbaum (2016). "Neuroendocrine Regulation of Growth Hormone Secretion." Compr Physiol **6**(2): 687-735.
Storey, N. M., S. Gentile, H. Ullah, A. Russo, M. Muessel, C. Erxleben and D. L. Armstrong (2006). "Rapid signaling at the plasma membrane by a nuclear receptor for thyroid hormone." Proc Natl Acad Sci U S A **103**(13): 5197-5201.
Stott, D. J., N. Rodondi, P. M. Kearney, I. Ford, R. G. J. Westendorp, S. P. Mooijaart, N. Sattar, C. E. Aubert, D. Aujesky, D. C. Bauer, C. Baumgartner, M. R. Blum, J. P. Browne, S. Byrne, T. H. Collet, O. M. Dekkers, W. P. J. den Elzen, R. S. Du Puy, G. Ellis, M. Feller, C. Floriani, K. Hendry, C. Hurley, J. W. Jukema, S. Kean, M. Kelly, D. Krebs, P. Langhorne, G. McCarthy, V. McCarthy, A. McConnachie, M. McDade, M. Messow, A. O'Flynn, D. O'Riordan, R. K. E. Poortvliet, T. J. Quinn, A. Russell, C. Sinnott, J. W. A. Smit, H. A. Van Dorland, K. A. Walsh, E. K. Walsh, T. Watt, R. Wilson, J. Gussekloo and T. S. Group (2017). "Thyroid Hormone Therapy for Older Adults with Subclinical Hypothyroidism." N Engl J Med **376**(26): 2534-2544.

Stuss, D. T., D. Floden, M. P. Alexander, B. Levine and D. Katz (2001). "Stroop performance in focal lesion patients: dissociation of processes and frontal lobe lesion location." Neuropsychologia **39**(8): 771-786.
Suh, Y., G. Atzmon, M. O. Cho, D. Hwang, B. Liu, D. J. Leahy, N. Barzilai and P. Cohen (2008). "Functionally significant insulin-like growth factor I receptor mutations in centenarians." Proc Natl Acad Sci U S A **105**(9): 3438-3442.
Sun, L. Y., Y. Fang, A. Patki, J. J. Koopman, D. B. Allison, C. M. Hill, M. M. Masternak, J. Darcy, J. Wang, S. McFadden and A. Bartke (2017). "Longevity is impacted by growth hormone action during early postnatal period." Elife **6**.
Surks, M. I. and L. Boucai (2010). "Age- and race-based serum thyrotropin reference limits." J Clin Endocrinol Metab **95**(2): 496-502.
Surks, M. I. and J. G. Hollowell (2007). "Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism." J Clin Endocrinol Metab **92**(12): 4575-4582.
Svensson, J., K. Sjogren, J. Faldt, N. Andersson, O. Isaksson, J. O. Jansson and C. Ohlsson (2011). "Liver-derived IGF-I regulates mean life span in mice." PLoS One **6**(7): e22640.
Swaab, D. F. and A. M. Bao (2011). "(Re-)activation of neurons in aging and dementia: lessons from the hypothalamus." Exp Gerontol **46**(2-3): 178-184.
Swaab, D. F., F. C. Raadsheer, E. Endert, M. A. Hofman, W. Kamphorst and R. Ravid (1994). "Increased cortisol levels in aging and Alzheimer's disease in postmortem cerebrospinal fluid." J Neuroendocrinol **6**(6): 681-687.
Szlejf, C., C. K. Suemoto, I. S. Santos, P. A. Lotufo, M. F. Haueisen Sander Diniz, S. M. Barreto and I. M. Bensenor (2018). "Thyrotropin level and cognitive performance: Baseline results from the ELSA-Brasil Study." Psychoneuroendocrinology **87**: 152-158.
Talbot, K., H. Y. Wang, H. Kazi, L. Y. Han, K. P. Bakshi, A. Stucky, R. L. Fuino, K. R. Kawaguchi, A. J. Samoyedny, R. S. Wilson, Z. Arvanitakis, J. A. Schneider, B. A. Wolf, D. A. Bennett, J. Q. Trojanowski and S. E. Arnold (2012). "Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline." J Clin Invest **122**(4): 1316-1338.
Tan, Z. S., A. Beiser, R. S. Vasan, R. Au, S. Auerbach, D. P. Kiel, P. A. Wolf and S. Seshadri (2008). "Thyroid function and the risk of Alzheimer disease: the Framingham Study." Arch Intern Med **168**(14): 1514-1520.
Taniguchi, C. M., B. Emanuelli and C. R. Kahn (2006). "Critical nodes in signalling pathways: insights into insulin action." Nat Rev Mol Cell Biol **7**(2): 85-96.
Taormina, G., F. Ferrante, S. Vieni, N. Grassi, A. Russo and M. G. Mirisola (2019). "Longevity: Lesson from Model Organisms." Genes (Basel) **10**(7).
Tasker, J. G. and J. P. Herman (2011). "Mechanisms of rapid glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis." Stress **14**(4): 398-406.
Tatar, M., A. Bartke and A. Antebi (2003). "The endocrine regulation of aging by insulin-like signals." Science **299**(5611): 1346-1351.
Tazearslan, C., J. Huang, N. Barzilai and Y. Suh (2011). "Impaired IGF1R signaling in cells expressing longevity-associated human IGF1R alleles." Aging Cell **10**(3): 551-554.
Tessner, K. D., E. F. Walker, S. H. Dhruv, K. Hochman and S. Hamann (2007). "The relation of cortisol levels with hippocampus volumes under baseline and challenge conditions." Brain Res **1179**: 70-78.
Thornton, P. L., R. L. Ingram and W. E. Sonntag (2000). "Chronic [D-Ala2]-growth hormone-releasing hormone administration attenuates age-related deficits in spatial memory." J Gerontol A Biol Sci Med Sci **55**(2): B106-112.

Tomasi, D. and N. D. Volkow (2012). "Aging and functional brain networks." Mol Psychiatry **17**(5): 471, 549-458.
Tordjman, K., A. Jaffe, Y. Trostanetsky, Y. Greenman, R. Limor and N. Stern (2000). "Low-dose (1 microgram) adrenocorticotrophin (ACTH) stimulation as a screening test for impaired hypothalamo-pituitary-adrenal axis function: sensitivity, specificity and accuracy in comparison with the high-dose (250 microgram) test." Clin Endocrinol (Oxf) **52**(5): 633-640.
Torres Aleman, I. (2012). "Insulin-like growth factor-1 and central neurodegenerative diseases." Endocrinol Metab Clin North Am **41**(2): 395-408, vii.
Trueba-Saiz, A., C. Cavada, A. M. Fernandez, T. Leon, D. A. Gonzalez, J. Fortea Ormaechea, A. Lleo, T. Del Ser, A. Nunez and I. Torres-Aleman (2013). "Loss of serum IGF-I input to the brain as an early biomarker of disease onset in Alzheimer mice." Transl Psychiatry **3**: e330.
Tsai, M. J. and B. W. O'Malley (1994). "Molecular mechanisms of action of steroid/thyroid receptor superfamily members." Annu Rev Biochem **63**: 451-486.
Tucker, A. M. and Y. Stern (2011). "Cognitive reserve in aging." Curr Alzheimer Res **8**(4): 354-360.
Tulving, E. (2001). "Episodic memory and common sense: how far apart?" Philos Trans R Soc Lond B Biol Sci **356**(1413): 1505-1515.
Tumati, S., H. Burger, S. Martens, Y. T. van der Schouw and A. Aleman (2016). "Association between Cognition and Serum Insulin-Like Growth Factor-1 in Middle-Aged & Older Men: An 8 Year Follow-Up Study." PLoS One **11**(4): e0154450.
Turner, K. M., J. W. Young, J. J. McGrath, D. W. Eyles and T. H. Burne (2013). "Cognitive performance and response inhibition in developmentally vitamin D (DVD)-deficient rats." Behav Brain Res **242**: 47-53.
Turpeinen, U. and E. Hamalainen (2013). "Determination of cortisol in serum, saliva and urine." Best Pract Res Clin Endocrinol Metab **27**(6): 795-801.
Ugalde, A. P., Y. Espanol and C. Lopez-Otin (2011). "Micromanaging aging with miRNAs: new messages from the nuclear envelope." Nucleus **2**(6): 549-555.
United Nations, D. o. E. a. S. A., Population Division (2017). World Population Prospects: The 2017 Revision, Volume II: Demographic Profiles (ST/ESA/SER.A/400). New York, United Nations.
Vadiveloo, T., P. T. Donnan, L. Cochrane and G. P. Leese (2011). "The Thyroid Epidemiology, Audit, and Research Study (TEARS): the natural history of endogenous subclinical hyperthyroidism." J Clin Endocrinol Metab **96**(1): E1-8.
Vadiveloo, T., P. T. Donnan, M. J. Murphy and G. P. Leese (2013). "Age- and gender-specific TSH reference intervals in people with no obvious thyroid disease in Tayside, Scotland: the Thyroid Epidemiology, Audit, and Research Study (TEARS)." J Clin Endocrinol Metab **98**(3): 1147-1153.
Vaiserman, A. M. (2014). "Early-life nutritional programming of longevity." J Dev Orig Health Dis **5**(5): 325-338.
van Boxtel, M. P., P. P. Menheere, O. Bekers, E. Hogervorst and J. Jolles (2004). "Thyroid function, depressed mood, and cognitive performance in older individuals: the Maastricht Aging Study." Psychoneuroendocrinology **29**(7): 891-898.
van Bunderen, C. C., J. B. Deijen and M. L. Drent (2018). "Effect of low-normal and high-normal IGF-1 levels on memory and wellbeing during growth hormone replacement therapy: a randomized clinical trial in adult growth hormone deficiency." Health Qual Life Outcomes **16**(1): 135.
van Bunderen, C. C., I. C. van Nieuwpoort, N. M. van Schoor, D. J. Deeg, P. Lips and M. L. Drent (2010). "The association of serum insulin-like growth factor-I with mortality, cardiovascular disease, and cancer in the elderly: a population-based study." J Clin Endocrinol Metab **95**(10): 4616-4624.
Van Cauter, E., R. Leproult and D. J. Kupfer (1996). "Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol." J Clin Endocrinol Metab **81**(7): 2468-2473.

van Dam, P. S., C. F. de Winter, R. de Vries, J. van der Grond, M. L. Drent, M. Lijffijt, J. L. Kenemans, A. Aleman, E. H. de Haan and H. P. Koppeschaar (2005). "Childhood-onset growth hormone deficiency, cognitive function and brain N-acetylaspartate." Psychoneuroendocrinology **30**(4): 357-363.
van den Beld, A. W., J. M. Kaufman, M. C. Zillikens, S. W. J. Lamberts, J. M. Egan and A. J. van der Lely (2018). "The physiology of endocrine systems with ageing." Lancet Diabetes Endocrinol **6**(8): 647-658.
van der Schaft, J., H. L. Koek, E. Dijkstra, H. J. Verhaar, Y. T. van der Schouw and M. H. Emmelot-Vonk (2013). "The association between vitamin D and cognition: a systematic review." Ageing Res Rev **12**(4): 1013-1023.
van der Spoel, E., S. W. Jansen, A. A. Akintola, B. E. Ballieux, C. M. Cobbaert, P. E. Slagboom, G. J. Blauw, R. G. J. Westendorp, H. Pijl, F. Roelfsema and D. van Heemst (2016). "Growth hormone secretion is diminished and tightly controlled in humans enriched for familial longevity." Aging Cell **15**(6): 1126-1131.
van der Spoel, E., M. P. Rozing, J. J. Houwing-Duistermaat, P. E. Slagboom, M. Beekman, A. J. de Craen, R. G. Westendorp and D. van Heemst (2015). "Association analysis of insulin-like growth factor-1 axis parameters with survival and functional status in nonagenarians of the Leiden Longevity Study." Aging (Albany NY) **7**(11): 956-963.
Vannucchi, G., D. Covelli, B. Vigo, M. Perrino, L. Mondina and L. Fugazzola (2017). "Thyroid volume and serum calcitonin changes during pregnancy." J Endocrinol Invest **40**(7): 727-732.
Vaz, L. J., M. Pradella-Hallinan, O. F. Bueno and S. Pompeia (2011). "Acute glucocorticoid effects on the multicomponent model of working memory." Hum Psychopharmacol **26**(7): 477-487.
Veldhuis, J. D. (2013). "Changes in pituitary function with ageing and implications for patient care." Nat Rev Endocrinol **9**(4): 205-215.
Veldhuis, J. D., C. Norman, J. M. Miles and C. Y. Bowers (2012). "Sex steroids, GHRH, somatostatin, IGF-I, and IGFBP-1 modulate ghrelin's dose-dependent drive of pulsatile GH secretion in healthy older men." J Clin Endocrinol Metab **97**(12): 4753-4760.
Veldhuis, J. D., A. Sharma and F. Roelfsema (2013). "Age-dependent and gender-dependent regulation of hypothalamic-adrenocorticotropic-adrenal axis." Endocrinol Metab Clin North Am **42**(2): 201-225.
Verhaeghen, P. (2013). Cognitive Aging, Oxford University Press.
Verhaeghen, P. and J. Cerella (2002). "Aging, executive control, and attention: a review of meta-analyses." Neurosci Biobehav Rev **26**(7): 849-857.
Villeda, S. A., K. E. Plambeck, J. Middeldorp, J. M. Castellano, K. I. Mosher, J. Luo, L. K. Smith, G. Bieri, K. Lin, D. Berdnik, R. Wabl, J. Udeochu, E. G. Wheatley, B. Zou, D. A. Simmons, X. S. Xie, F. M. Longo and T. Wyss-Coray (2014). "Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice." Nat Med **20**(6): 659-663.
Vitale, G., S. Salvioli and C. Franceschi (2013). "Oxidative stress and the ageing endocrine system." Nat Rev Endocrinol **9**(4): 228-240.
Vitiello, M. V., K. E. Moe, G. R. Merriam, G. Mazzoni, D. H. Buchner and R. S. Schwartz (2006). "Growth hormone releasing hormone improves the cognition of healthy older adults." Neurobiol Aging **27**(2): 318-323.
Vogel, A., T. V. Elberling, M. Hording, J. Dock, A. K. Rasmussen, U. Feldt-Rasmussen, H. Perrild and G. Waldemar (2007). "Affective symptoms and cognitive functions in the acute phase of Graves' thyrotoxicosis." Psychoneuroendocrinology **32**(1): 36-43.
Vollmann-Honsdorf, G. K., G. Flugge and E. Fuchs (1997). "Chronic psychosocial stress does not affect the number of pyramidal neurons in tree shrew hippocampus." Neurosci Lett **233**(2-3): 121-124.

Volpato, S., J. M. Guralnik, L. P. Fried, A. T. Remaley, A. R. Cappola and L. J. Launer (2002). "Serum thyroxine level and cognitive decline in euthyroid older women." Neurology **58**(7): 1055-1061.
Wager, T. D. and E. E. Smith (2003). "Neuroimaging studies of working memory: a meta-analysis." Cogn Affect Behav Neurosci **3**(4): 255-274.
Wahlin, A., D. Bunce and T. B. Wahlin (2005). "Longitudinal evidence of the impact of normal thyroid stimulating hormone variations on cognitive functioning in very old age." Psychoneuroendocrinology **30**(7): 625-637.
Wahlin, A., T. B. Wahlin, B. J. Small and L. Backman (1998). "Influences of thyroid stimulating hormone on cognitive functioning in very old age." J Gerontol B Psychol Sci Soc Sci **53**(4): P234-239.
Wallace, A. M., S. Gibson, A. de la Hunty, C. Lamberg-Allardt and M. Ashwell (2010). "Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations." Steroids **75**(7): 477-488.
Walsh, J. P., L. C. Ward, V. Burke, C. I. Bhagat, L. Shiels, D. Henley, M. J. Gillett, R. Gilbert, M. Tanner and B. G. Stuckey (2006). "Small changes in thyroxine dosage do not produce measurable changes in hypothyroid symptoms, well-being, or quality of life: results of a double-blind, randomized clinical trial." J Clin Endocrinol Metab **91**(7): 2624-2630.
Walshaw, P. D., L. Gyulai, M. Bauer, M. S. Bauer, B. Calimlim, C. A. Sugar and P. C. Whybrow (2018). "Adjunctive thyroid hormone treatment in rapid cycling bipolar disorder: A double-blind placebo-controlled trial of levothyroxine (L-T4) and triiodothyronine (T3)." Bipolar Disord **20**(7): 594-603.
Wang, Y., J. Zhu and H. F. DeLuca (2012). "Where is the vitamin D receptor?" Arch Biochem Biophys **523**(1): 123-133.
Waring, A. C., A. M. Arnold, A. B. Newman, P. Buzkova, C. Hirsch and A. R. Cappola (2012). "Longitudinal changes in thyroid function in the oldest old and survival: the cardiovascular health study all-stars study." J Clin Endocrinol Metab **97**(11): 3944-3950.
Watson, S., P. Gallagher, R. J. Porter, M. S. Smith, L. J. Herron, S. Bulmer, G. North-East Mood Disorders Clinical Research, A. H. Young and I. N. Ferrier (2012). "A randomized trial to examine the effect of mifepristone on neuropsychological performance and mood in patients with bipolar depression." Biol Psychiatry **72**(11): 943-949.
Webb, E. A., M. A. O'Reilly, J. D. Clayden, K. K. Seunarine, W. K. Chong, N. Dale, A. Salt, C. A. Clark and M. T. Dattani (2012). "Effect of growth hormone deficiency on brain structure, motor function and cognition." Brain **135**(Pt 1): 216-227.
Weltman, A., J. Y. Weltman, C. P. Roy, L. Wideman, J. Patrie, W. S. Evans and J. D. Veldhuis (2006). "Growth hormone response to graded exercise intensities is attenuated and the gender difference abolished in older adults." J Appl Physiol (1985) **100**(5): 1623-1629.
Wennberg, A. M. V., C. E. Hagen, M. M. Machulda, J. H. Hollman, R. O. Roberts, D. S. Knopman, R. C. Petersen and M. M. Mielke (2018). "The association between peripheral total IGF-1, IGFBP-3, and IGF-1/IGFBP-3 and functional and cognitive outcomes in the Mayo Clinic Study of Aging." Neurobiol Aging **66**: 68-74.
Werner, H., D. Weinstein and I. Bentov (2008). "Similarities and differences between insulin and IGF-I: structures, receptors, and signalling pathways." Arch Physiol Biochem **114**(1): 17-22.
West, M. J., P. D. Coleman, D. G. Flood and J. C. Troncoso (1994). "Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease." Lancet **344**(8925): 769-772.

Westwood, A. J., A. Beiser, C. Decarli, T. B. Harris, T. C. Chen, X. M. He, R. Roubenoff, A. Pikula, R. Au, L. E. Braverman, P. A. Wolf, R. S. Vasan and S. Seshadri (2014). "Insulin-like growth factor-1 and risk of Alzheimer dementia and brain atrophy." Neurology **82**(18): 1613-1619.
Wheeler, H. E. and S. K. Kim (2011). "Genetics and genomics of human ageing." Philos Trans R Soc Lond B Biol Sci **366**(1561): 43-50.
Wheeler, S. M., M. P. McAndrews, E. D. Sheard and J. Rovet (2012). "Visuospatial associative memory and hippocampal functioning in congenital hypothyroidism." J Int Neuropsychol Soc **18**(1): 49-56.
Wheeler, S. M., V. C. McLelland, E. Sheard, M. P. McAndrews and J. F. Rovet (2015). "Hippocampal Functioning and Verbal Associative Memory in Adolescents with Congenital Hypothyroidism." Front Endocrinol (Lausanne) **6**: 163.
Wheeler, S. M., K. A. Willoughby, M. P. McAndrews and J. F. Rovet (2011). "Hippocampal size and memory functioning in children and adolescents with congenital hypothyroidism." J Clin Endocrinol Metab **96**(9): E1427-1434.
WHO (2002). Active Ageing: A Policy Framework, World Health Organization.
WHO (2015). World report on ageing and health. Geneva, World Health Organization.
WHO, National Institute on Aging, National Institutes of Health and U. S. D. o. H. a. H. Services (2011). Global Health and Aging, NIH Publication no. 11-7737, National Institutes of Health.
WHO, UNICEF and ICCIDD (2007). "Assessment of iodine deficiency disorders and monitoring their elimination : a guide for programme managers." WHO **Geneva**.
Wijsman, L. W., A. J. de Craen, S. Trompet, J. Gussekloo, D. J. Stott, N. Rodondi, P. Welsh, J. W. Jukema, R. G. Westendorp and S. P. Mooijaart (2013). "Subclinical thyroid dysfunction and cognitive decline in old age." PLoS One **8**(3): e59199.
Wilhelm, I., J. Born, B. M. Kudielka, W. Schlotz and S. Wust (2007). "Is the cortisol awakening rise a response to awakening?" Psychoneuroendocrinology **32**(4): 358-366.
Wilkinson, C. W., E. R. Peskind and M. A. Raskind (1997). "Decreased hypothalamic-pituitary-adrenal axis sensitivity to cortisol feedback inhibition in human aging." Neuroendocrinology **65**(1): 79-90.
Wilkinson, J. E., L. Burmeister, S. V. Brooks, C. C. Chan, S. Friedline, D. E. Harrison, J. F. Hejtmancik, N. Nadon, R. Strong, L. K. Wood, M. A. Woodward and R. A. Miller (2012). "Rapamycin slows aging in mice." Aging Cell **11**(4): 675-682.
Williams, G. C. (1957). "Pleiotropy, Natural Selection, and the Evolution of Senescence." Evolution **11**(4): 398-411.
Willoughby, K. A., M. P. McAndrews and J. F. Rovet (2014). "Effects of maternal hypothyroidism on offspring hippocampus and memory." Thyroid **24**(3): 576-584.
Wingenfeld, K., S. Wolf, J. C. Krieg and S. Lautenbacher (2011). "Working memory performance and cognitive flexibility after dexamethasone or hydrocortisone administration in healthy volunteers." Psychopharmacology (Berl) **217**(3): 323-329.
Wingfield, A., P. A. Tun, C. K. Koh and M. J. Rosen (1999). "Regaining lost time: adult aging and the effect of time restoration on recall of time-compressed speech." Psychol Aging **14**(3): 380-389.
Woelfle, J., D. J. Chia and P. Rotwein (2003). "Mechanisms of growth hormone (GH) action. Identification of conserved Stat5 binding sites that mediate GH-induced insulin-like growth factor-I gene activation." J Biol Chem **278**(51): 51261-51266.
Wolf, O. T., P. Atsak, D. J. de Quervain, B. Roozendaal and K. Wingenfeld (2016). "Stress and Memory: A Selective Review on Recent Developments in the Understanding of Stress Hormone Effects on Memory and Their Clinical Relevance." J Neuroendocrinol **28**(8).

Woods, K. A., C. Camacho-Hubner, M. O. Savage and A. J. Clark (1996). "Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene." N Engl J Med **335**(18): 1363-1367.
Wrutniak-Cabello, C., F. Casas and G. Cabello (2001). "Thyroid hormone action in mitochondria." J Mol Endocrinol **26**(1): 67-77.
Wu, Y., Y. Pei, F. Wang, D. Xu and W. Cui (2016). "Higher FT4 or TSH below the normal range are associated with increased risk of dementia: a meta-analysis of 11 studies." Sci Rep **6**: 31975.
Wullschleger, S., R. Loewith and M. N. Hall (2006). "TOR signaling in growth and metabolism." Cell **124**(3): 471-484.
Yamamoto, H., K. Schoonjans and J. Auwerx (2007). "Sirtuin functions in health and disease." Mol Endocrinol **21**(8): 1745-1755.
Yang, S., A. Megill, A. O. Ardiles, S. Ransom, T. Tran, M. T. Koh, H. K. Lee, M. Gallagher and A. Kirkwood (2013). "Integrity of mGluR-LTD in the associative/commissural inputs to CA3 correlates with successful aging in rats." J Neurosci **33**(31): 12670-12678.
Yeap, B. B., H. Alfonso, S. A. Chubb, G. Puri, G. J. Hankey, L. Flicker and O. P. Almeida (2012). "Higher free thyroxine levels predict increased incidence of dementia in older men: the Health in Men Study." J Clin Endocrinol Metab **97**(12): E2230-2237.
Yin, J. J., L. M. Liao, D. X. Luo, K. Xu, S. H. Ma, Z. X. Wang, H. B. Le, R. R. Huang, Z. L. Cai and J. Zhang (2013). "Spatial working memory impairment in subclinical hypothyroidism: an FMRI study." Neuroendocrinology **97**(3): 260-270.
Yoshiya, M., Y. Komatsuzaki, Y. Hojo, M. Ikeda, H. Mukai, Y. Hatanaka, G. Murakami, M. Kawata, T. Kimoto and S. Kawato (2013). "Corticosterone rapidly increases thorns of CA3 neurons via synaptic/extranuclear glucocorticoid receptor in rat hippocampus." Front Neural Circuits **7**: 191.
Young, A. H., B. J. Sahakian, T. W. Robbins and P. J. Cowen (1999). "The effects of chronic administration of hydrocortisone on cognitive function in normal male volunteers." Psychopharmacology (Berl) **145**(3): 260-266.
Yuen, E. Y., W. Liu, I. N. Karatsoreos, J. Feng, B. S. McEwen and Z. Yan (2009). "Acute stress enhances glutamatergic transmission in prefrontal cortex and facilitates working memory." Proc Natl Acad Sci U S A **106**(33): 14075-14079.
Zaia, A., P. Maponi, G. Di Stefano and T. Casoli (2017). "Biocomplexity and Fractality in the Search of Biomarkers of Aging and Pathology: Focus on Mitochondrial DNA and Alzheimer's Disease." Aging Dis **8**(1): 44-56.
Zanto, T. P. and A. Gazzaley (2014). Attention and aging. The Oxford handbook of attention. A. C. N. a. S. Kastner, Oxford University Press**:** 927-971.
Zec, R. F., S. J. Markwell, N. R. Burkett and D. L. Larsen (2005). "A longitudinal study of confrontation naming in the "normal" elderly." J Int Neuropsychol Soc **11**(6): 716-726.
Zehnder, D., R. Bland, M. C. Williams, R. W. McNinch, A. J. Howie, P. M. Stewart and M. Hewison (2001). "Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase." J Clin Endocrinol Metab **86**(2): 888-894.
Zelazo, P. D., F. I. Craik and L. Booth (2004). "Executive function across the life span." Acta Psychol (Amst) **115**(2-3): 167-183.
Zhai, Q., Z. Lai, P. Roos and F. Nyberg (1994). "Characterization of growth hormone binding sites in rat brain." Acta Paediatr Suppl **406**: 92-95.
Zhang, H., M. Han, X. Zhang, X. Sun and F. Ling (2014). "The effect and mechanism of growth hormone replacement on cognitive function in rats with traumatic brain injury." PLoS One **9**(9): e108518.
Zhang, H., K. J. Menzies and J. Auwerx (2018). "The role of mitochondria in stem cell fate and aging." Development **145**(8).

Zhang, W., X. Liu, Y. Zhang, L. Song, J. Hou, B. Chen, M. He, P. Cai and H. Lii (2014). "Disrupted functional connectivity of the hippocampus in patients with hyperthyroidism: evidence from resting-state fMRI." Eur J Radiol **83**(10): 1907-1913.
Zhang, W., L. Song, X. Yin, J. Zhang, C. Liu, J. Wang, D. Zhou, B. Chen and H. Lii (2014). "Grey matter abnormalities in untreated hyperthyroidism: a voxel-based morphometry study using the DARTEL approach." Eur J Radiol **83**(1): e43-48.
Zhu, D. F., Z. X. Wang, D. R. Zhang, Z. L. Pan, S. He, X. P. Hu, X. C. Chen and J. N. Zhou (2006). "fMRI revealed neural substrate for reversible working memory dysfunction in subclinical hypothyroidism." Brain **129**(Pt 11): 2923-2930.
Zhu, H., N. Wang, L. Yao, Q. Chen, R. Zhang, J. Qian, Y. Hou, W. Guo, S. Fan, S. Liu, Q. Zhao, F. Du, X. Zuo, Y. Guo, Y. Xu, J. Li, T. Xue, K. Zhong, X. Song, G. Huang and W. Xiong (2018). "Moderate UV Exposure Enhances Learning and Memory by Promoting a Novel Glutamate Biosynthetic Pathway in the Brain." Cell **173**(7): 1716-1727 e1717.
Zhu, W., Y. Fan, T. Frenzel, M. Gasmi, R. T. Bartus, W. L. Young, G. Y. Yang and Y. Chen (2008). "Insulin growth factor-1 gene transfer enhances neurovascular remodeling and improves long-term stroke outcome in mice." Stroke **39**(4): 1254-1261.
Zimmermann, M. B. and M. Andersson (2012). "Assessment of iodine nutrition in populations: past, present, and future." Nutr Rev **70**(10): 553-570.
Zimmermann, M. B. and K. Boelaert (2015). "Iodine deficiency and thyroid disorders." Lancet Diabetes Endocrinol **3**(4): 286-295.
Zimmermann, M. B., K. Connolly, M. Bozo, J. Bridson, F. Rohner and L. Grimci (2006). "Iodine supplementation improves cognition in iodine-deficient schoolchildren in Albania: a randomized, controlled, double-blind study." Am J Clin Nutr **83**(1): 108-114.
Zivadinov, R., C. N. Treu, B. Weinstock-Guttman, C. Turner, N. Bergsland, K. O'Connor, M. G. Dwyer, E. Carl, D. P. Ramasamy, J. Qu and M. Ramanathan (2013). "Interdependence and contributions of sun exposure and vitamin D to MRI measures in multiple sclerosis." J Neurol Neurosurg Psychiatry **84**(10): 1075-1081.
Zoeller, R. T. and J. Rovet (2004). "Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings." J Neuroendocrinol **16**(10): 809-818.

9. Annex

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
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25-OH Vitamin D Levels and Cognitive Performance: Longitudinal Assessment in a Healthy Aging Cohort

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Background: Declining serum levels of 25-hydroxyvitamin D [25(OH)D, a biomarker of vitamin D status] with aging is a well-recognized phenomenon. However, scarce information is available on the relation between 25(OH)D levels and cognitive performance over time in older individuals. Our purpose was to evaluate, longitudinally, the association of 25(OH)D with cognitive function in a healthy older adults' cohort.

Methods: Sixty-four individuals over 55 years-old with no cognitive impairment, clustered as healthy "Poor" and "Good" cognitive performers, were followed for an average of 18 months. Seasonal-adjusted 25(OH)D serum levels (measured by high-performance liquid chromatography-tandem mass spectrometry) were related, longitudinally, with cognitive (memory and general/executive) composite scores.

Results: Overall seasonal-adjusted median serum 25(OH)D level was of 47 nmol/l [interquartile range (IQR), 38–60 nmol/l]. A negative correlation between baseline 25(OH)D and the general/executive composite score was found in the "Poor" cognitive performers ($r^S = -0.52$, $p = 0.006$), an association lost after adjusting 25(OH)D levels for the season. No effect was found in both groups between seasonal-adjusted 25(OH)D levels and the variation of both memory and general/executive composites during follow-up when adjusted for age, gender and education level.

Conclusion: In this healthy older population with no cognitive impairment, lower serum levels of 25(OH)D were not longitudinally associated with poorer cognitive scores.

Keywords: 25-hydroxyvitamin D, vitamin D, aging, healthy aging, cognitive performance, longitudinal analysis

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INTRODUCTION

Vitamin D is a steroid prohormone obtained from the diet or produced by the action of ultraviolet light in the skin (Bouillon, 2016). Once in circulation, it is rapidly hydroxylated in the liver into 25-OH vitamin D [25(OH)D]. Levels of 25(OH)D are considered surrogate indicators of vitamin D homeostasis (Bouillon, 2016). While its best well-known function resides

on the regulation of calcium homeostasis, evidence is accumulating on the association of vitamin D deficiency with reduced musculoskeletal health and increased risk for acute and chronic diseases, as well as all-cause mortality (Pludowski et al., 2013; Schöttker et al., 2014). More so, some studies found significant positive associations between blood 25(OH)D and several cognitive performance scores in different gender and age groups (Annweiler et al., 2010; van der Schaft et al., 2013; Anastasiou et al., 2014; Granic et al., 2015). However, available observational data in older populations, evaluating various cognition domains and 25(OH)D levels provided contradictory results. Several, but not all, cross-sectional and prospective studies involving adults older than 60 years have shown an increased risk of cognitive impairment for those with low levels of 25(OH)D (Annweiler et al., 2013; Goodwill et al., 2018). Still, overall, very few studies have addressed this issue longitudinally in a community-dwelling senior population setting and with a standardized 25(OH)D serum measurement method (Perna et al., 2014; Kuźma et al., 2016).

Here the objective was to evaluate the association between cognitive longitudinal performance (average follow-up of 18 months) and 25(OH)D serum levels determined by high-performance liquid chromatography-tandem mass spectrometry. The population sample was comprised of healthy individuals aged 55 years and older, with no cognitive impairment, but characterized by distinct "normal" cognitive performance patterns.

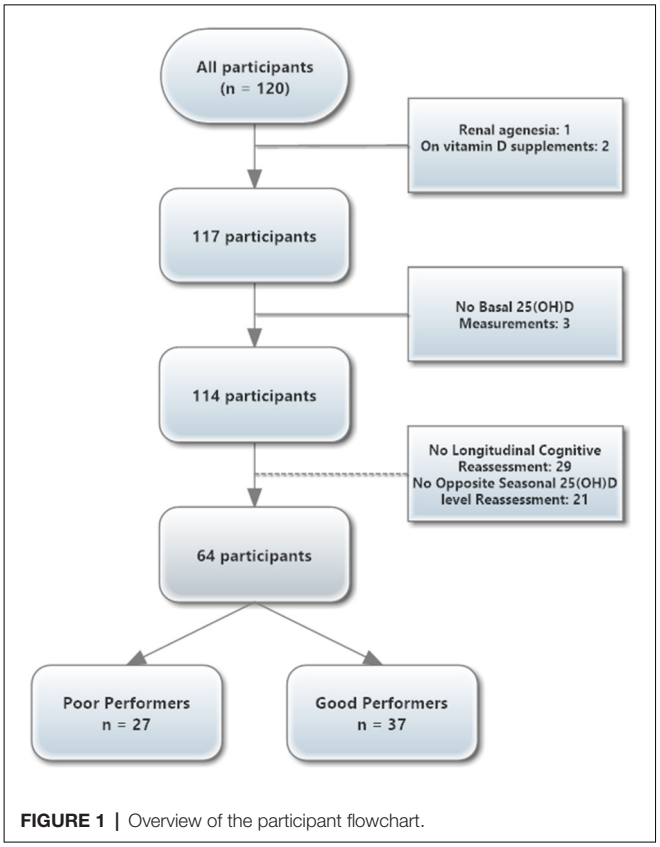

FIGURE 1 | Overview of the participant flowchart.

MATERIALS AND METHODS

Subjects

The study was conducted between March 2012 and March 2015. Summarily, the recruitment was performed in two-phases. First, a larger sample, representative of the general Portuguese older population in terms of age, gender, and education underwent a full neuropsychological assessment (subjects were randomly selected from the Guimarães and Vizela local area health authority registries), resulting in 1,051 participants after inclusion/exclusion criteria (Costa et al., 2013; Santos et al., 2013, 2014). Then, of these, 120 subjects (matched for gender and age) were chosen in order to provide cognitive profiles of overall "good" cognitive performance (n = 60) and overall "poor" performance (n = 60) group, based on their, within normal range, neuropsychological testing. Primary exclusion criteria included inability to understand informed consent, participant choice to withdraw from the study, dementia and/or diagnosed neuropsychiatric and/or neurodegenerative disorder. Adjusted thresholds for cognitive impairment were calculated depending on factors such as age and/or education (Grigoletto et al., 1999; Busch and Chapin, 2008). Thus, the Mini-Mental State Examination (MMSE) test score thresholds applied were the following: MMSE score <17, if individual with 4 or less years of formal school education and/or 72 or more years of age, and MMSE score <23 otherwise (follows the MMSE validation study for the Portuguese population; Guerreiro et al., 1994). All participants were community dwellers. For final analysis purposes, subjects with prior history of renal failure, cerebrovascular disorders, osteomalacia, any other bone disease, or those who were on calcium and/or vitamin D supplements were excluded. Individuals who presented estimated glomerular filtration rate below 50 ml/min/1.73m^2 and/or who did not have 25(OH)D serum concentration data available were also excluded. The final sample for consideration to the longitudinal analysis was of 64 participants, all of them attended the complete evaluation sessions and had a "seasonal-adjusted" vitamin D evaluation (**Figure 1**).

The study was conducted in accordance with the Declaration of Helsinki and approved by national and local ethics review boards. All study goals and nature of the tests were explained to the potential participants and informed signed consent obtained.

Analytical Methods

The season of blood collection was dichotomized into Winter-Spring (between 21st December and 20th June) and Summer-Autumn (between 21st June and 20th December), based on the usual solar solstices/equinoxes' dates. All subjects underwent fasting for morning blood collection. Blood samples were collected, centrifuged and stored at −20°C until 25(OH)D levels determination. A seasonal-adjusted mean 25(OH)D levels were obtained for each participant with two blood samples performed on a different season collection (Winter-Spring and Summer-Autumn) over a 1–2 years period follow-up to create an individual "season-adjusted" value (n = 64). The 25(OH)D measurement was obtained by a high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS)

method (Waters Corporation, Milford, MA, USA) with a coefficient of variation of 4.9%. As expected (since individuals under supplementation were excluded from the study), 25(OH)D2 levels were negligible (corresponded to less than 1% of the full D2+D3 concentration). Therefore, total 25(OH)D levels reported here correspond to the 25(OH)D3 determination.

Vitamin D deficiency definition based on serum 25(OH)D values is not consensual. For description purposes, we categorized into three groups based on: 30 nmol/l (12 ng/ml), 50 nmol/l (20 ng/ml) and 75 nmol/l (30 ng/ml) 25(OH)D cut-point levels ("deficiency," "adequacy" and "optimal" thresholds, respectively; Holick et al., 2011).

Vitamin D Intake

Vitamin D intake was assessed by a 24 h diet recall questionnaire. This estimation was performed using the Nutrilog® software (Nutrilog SAS, France), resorting to the release 23 of the United States Department of Agriculture National Nutrient Database for Standard Reference and adapted to the Portuguese foods using the Portuguese Food Composition Database (INRJ, 2018). Food vitamin D fortification is not commonly practiced in Portugal (Barroso, 2014).

Cognitive Assessment

A team of trained psychologists performed the cognitive/neuropsychological assessments. A test battery was used for socio-demographic characterization and to evaluate multiple neuropsychological dimensions, including cognition profiles [general cognitive status and executive (EXEC) and memory (MEM) functions], as previously reported (Costa et al., 2013). Briefly, these included: Graffar socio-demographic scale, digit-span forward and backward test, Stroop color and word test, controlled oral word association test (COWAT), selective reminding test (SRT), digit symbol substitution test (DSST) and MMSE (scores adjusted for cognitive impairment and Portuguese population; Guerreiro et al., 1994). A Principal Component Analysis was performed in order to allocate the multiple test variables into composite components/dimensions, as previously reported (Santos et al., 2013). Summarily, this resulted in the identification of significant dimensions: memory (MEM; SRT test variables: consistent long-term retrieval, long-term storage and delayed recall) and general/executive function (EXEC; COWAT letters F-A-S admissible parameter; Stroop parameters: words, colors and words/colors, digits parameters: forward and backward; MMSE). A z-score for the cognitive composite was calculated and used to select normal extreme values and select "Poor" and "Good" cognitive performers. In our population, the suitability of using this battery of cognitive tests to measure two latent constructs, memory and executive functioning, was previously demonstrated by a longitudinal invariance analysis across the follow-up (Moreira et al., 2018).

Statistical Analysis

All data are presented as the mean (median), standard deviation (SD); inter-quartile-range (IQR) for normally (non-normally) distributed data. All continuous variables were checked for normality using the Shapiro-Wilk normality test. Unpaired *t*-test and Mann-Whitney *U*-test were used to compare continuous variables between the two groups, as appropriate. Wilcoxon matched-pairs signed-rank test was performed to compare paired variables that failed normality tests. Fisher's exact test was used for categorical variables. The confidence interval of a proportion was obtained by the modified Wald method. Univariate analysis with Spearman's rank correlation was performed to assess linear relationship between 25(OH)D levels and the different cognitive domain scores (MEM and EXEC) and its temporal trends, stratified by cognitive group. A multiple linear regression analysis was conducted to explore the association between seasonal-adjusted 25(OH)D levels and age, gender and vitamin D intake estimation. Similarly, a multiple linear regression analysis was done to evaluate the prediction of Memory/Executive function scores over time by seasonal-adjusted 25(OH)D levels, adjusted to age, gender, baseline cognitive group and education level. Effect size estimates were calculated with Cohen's d and η^2 for continuous variables with parametric and non-parametric comparisons, respectively; r for Wilcoxon matched paired test; ϕ coefficient for Fisher's exact test; and R^2 and adjusted R^2 for regression analysis.

All analyses were tested at the 0.05 level of significance and performed using IBM SPSS Statistics, v.21 (IBM, New York, NY, USA) and GraphPad Prism, v.6.00 (GraphPad Software, La Jolla, CA, USA). Effect size estimates were evaluated by Cohen's published benchmark classes (small, medium and large) with the following proposed cut-points: 0.2, 0.5 and 0.8 for Cohen's d; 0.01, 0.06, 0.14 for η^2; 0.1, 0.3 and 0.5 for r and ϕ coefficient; and 0.02, 0.13, 0.26 for R^2 (Cohen, 1988).

RESULTS

After exclusion criteria, the final sample included 64 individuals with a mean age of 65 years (SD 8 years), a women ratio of 0.47 (n = 30) and a median follow-up of 18 months (min–max: 16–22 months). Median 25(OH)D levels were higher during Summer-Fall when compared to Winter-Spring season (55 nmol/l vs. 43 nmol/l, $p = 0.01$, $\eta^2 = 0.06$). The overall median seasonal-adjusted serum 25(OH)D level was 47 nmol/l (IQR 38–60 nmol/l; range 13–114 nmol/l). Total study population characteristics are presented in **Table 1**. Overall vitamin D deficiency (below 30 nmol/l) was found in 10 subjects (16%, 95% CI 9–27%), with only seven participants (11%, 95% CI 5–21%) surpassing the threshold of optimal 25(OH)D serum concentration (≥75 nmol/L). Thirty-eight participants (59%, 95% CI 47–71%) had 25(OH)D levels below 50 nmol/l. These proportions were not different between "Good" and "Poor" groups (**Table 1**). The estimated median vitamin D intake was 0.9 μg (36 IU) per day [IQR 0.2–3.1 μg (8–124 IU) per day], and not different among cognitive groups (1.5 *vs.* 0.5 μg/day, $p = 0.06$), with a moderate/low practical effect size ($\eta^2 = 0.06$).

On univariate analysis, "Good" performers had higher education ($p = 0.004$, $\phi = 0.37$) when compared to "Poor" performers. Cognitive domain scores at baseline and final evaluation were different between the two groups ($p < 0.001$), with a high practical effect size ($\eta^2 = 0.72$), for both domains. MEM score tends to decrease over time in both groups

TABLE 1 | Study population characteristics by cognitive performance group, $n = 64$.

	Total ($n = 64$)	"Poor" ($n = 27$)	"Good" ($n = 37$)	p	Effect size
Socio-demographic features					
Sex					
Female, *n* (%)	30 (47)	14 (52)	16 (43)	0.61	0.09
Age, mean (SD), years	65 (8)	67 (7)	64 (9)	0.09	0.04
Education level above 4-years, *n* (%)	17 (27)	2 (8)	15 (41)	0.004	0.37
Vitamin D parameters					
Seasonal-adjusted median serum 25(OH)D concentration, nmol/l (IQR)	47 (38–60)	47 (40–54)	47 (36–61)	0.82	0.001
Vitamin D status [serum 25(OH)D levels]					
Below 30 nmol/l ("deficient"), *n* (%)	10 (16)	3 (11)	7 (19)	0.50	0.11
Below 50 nmol/l ("inadequate"), *n* (%)	38 (59)	16 (59)	22 (60)	>0.99	0.002
Between 50 and 74 nmol/l ("adequate"), *n* (%)	19 (30)	9 (33)	10 (27)	0.60	0.07
75 nmol/l or above ("optimal"), *n* (%)	7 (11)	2 (7)	5 (14)	0.69	0.01
Vitamin D intake estimation, μg/day, median (IQR)*	0.9 (0.2–3.1)	0.5 (0.1–2.3)	1.5 (0.2–5.6)	0.06	0.06
Cognitive scores and trends, Median (IQR)					
Baseline MEM score	0.23 (−0.97 to 0.74)	−0.98 (−1.27 to −0.73)	0.62 (0.35–0.98)	<0.001	0.72
Baseline EXEC score	0.09 (−0.84 to 0.81)	−0.89 (−1.05 to −0.72)	0.70 (0.41–1.27)	<0.001	0.72
Last MEM score	−0.65 (−1.19 to 0.49)	−1.16 (−1.49 to −0.91)	0.09 (−0.65 to 1.04)	<0.001	0.35
Last EXEC score	−0.17 (−0.94 to 0.89)	−0.96 (−1.26 to −0.55)	0.793 (0.45–1.31)	<0.001	0.71
MEM score Trend	−0.30 (−0.71 to 0.13)	−0.19 (−0.45 to 0.19)	−0.44 (−1.23 to 0.11)	0.03	0.04
EXEC score Trend	−0.004 (−0.11 to 0.14)	−0.003 (−0.12 to 0.16)	−0.009 (−0.10 to 0.14)	0.69	0.003

*IQR, interquartile range; MEM, memory dimension composite; EXEC, executive function dimension composite. *n = 63, 27 vs. 36.*

TABLE 2 | Spearman's rank correlation (r^s) between 25(OH)D levels and MEM and EXEC scores and its temporal trends (in both "Poor" and "Good" performers).

	"Poor" ($n = 27$)		"Good" ($n = 37$)	
	Baseline 25 (OH)D	Seasonal-adjusted 25 (OH)D	Baseline 25 (OH)D	Seasonal-adjusted 25 (OH)D
Baseline MEM score	−0.25	−0.06	0.31	0.25
Baseline EXEC score	−0.52[a]	−0.05	0.22	0.16
Last MEM score	−0.21	−0.05	0.20	0.16
Last EXEC score	−0.26	−0.01	0.11	0.08
MEM score trend	0.15	0.12	0.10	0.08
EXEC score trend	0.24	0.27	−0.13	−0.03

[a]$p = 0.006$.

but reached statistical significance only in the "Good" group ($p < 0.001$), with a very high practical effect size ($r = 0.56$). EXEC scores were stable ("Poor": $p = 0.83$, $r = 0.04$; "Good": $p = 0.53$, $r = 0.10$) and not different between "Poor" and "Good" groups ($p = 0.69$, $\eta^2 = 0.003$), with low practical effect size. There was no association between baseline 25(OH)D levels and MEM and EXEC scores (at baseline, last and longitudinal variation) in both groups, except for the first EXEC score obtained in the "Poor" performance group, with a high negative correlation score ($r^s = -0.52$, $p = 0.006$; **Table 2**). Seasonal-adjusted 25(OH)D levels were not correlated to any MEM and EXEC scores or to its longitudinal variation in both groups (**Table 2**).

To explore the association between the observed variations in MEM and EXEC scores over time with seasonal-adjusted 25(OH)D levels adjusted for baseline age, gender, cognitive performance and education level, a multiple linear regression analysis was performed (**Table 3**). In this fully adjusted model, 25(OH)D seasonal-adjusted serum concentration failed to predict cognitive performance over time (MEM score trend, $p = 0.96$, with moderate effect size: $R^2 = 0.19$, adjusted $R^2 = 0.12$; EXEC score trend, $p = 0.47$, with low effect size: $R^2 = 0.07$, adjusted $R^2 = -0.005$). Being a "Poor" performance individual was linked to a less detrimental decay in MEM score over time ($\beta = 0.68$, 95% CI 0.29–1.08, $p = 0.001$). No other variable evaluated showed a relevant effect on MEM or EXEC score trends (**Table 3**).

DISCUSSION

In this longitudinal study with a healthy non-demented older cohort, we found no association between standardized season-adjusted 25(OH)D levels and the variation of the "memory" and "executive" cognitive domains over a median follow-up of 18 months.

An important seasonal effect over cognitive performance is currently recognized, with annual variations in brain function reaching a peak in late Summer and early Fall and declining in late Winter and early Spring (Meyer et al., 2016; Lim et al., 2018). This observation may help explain some intraindividual cognitive changes that develop at specific times of the year and the importance of seasonal adjusting when exploring cognitive associations with other variables that share circannual cycles. We observed a negative correlation between baseline 25(OH)D values and baseline EXEC score in the "Poor" performers

TABLE 3 | Association between observed Memory/Executive Function scores trend with age, gender, cognitive group, education level and "seasonal-adjusted" 25(OH)D levels predicted by multiple linear regression analysis (n = 64; MEM score trend, p = 0.96, R^2 = 0.19, adjusted R^2 = 0.12; EXEC score trend, p = 0.47, R^2 = 0.07, adjusted R^2 = −0.005).

	MEM score trend		EXEC score trend	
	β (95% CI)	***p***	**β (95% CI)**	***p***
Age (years)	−0.01 (−1.95 to 1.82)	0.29	−0.002 (−0.01 to 0.006)	0.60
Gender (Exposure: Male)	−0.04 (−0.43 to 0.35)	0.84	−0.07 (−0.12 to 0.05)	0.25
Cognitive performance (Exposure: "Poor")	0.68 (0.29–1.08)	0.001	−0.01 (−0.14 to 0.11)	0.84
Education Level (years)	0.05 (−0.004 to 0.11)	0.07	0.01 (−0.005 to 0.03)	0.15
Seasonal-adjusted 25(OH)D levels (nmol/l)	0.001 (−0.01 to 0.01)	0.96	−0.001 (−0.004 to 0.002)	0.47

group, but this effect was lost after adjusting 25(OH)D for season. Our observations are in accordance with the few studies that evaluated cognitive performance and behavior in populations exclusively with vitamin D insufficiency like ours. Some authors using a very low 25(OH)D cut-point level (25 nmol/l), have shown a small or no clear positive association with cognitive performance (Aung et al., 2006; Annweiler et al., 2010). Others, using a somewhat higher 25(OH)D threshold levels, have reported better cognitive performance associated with these "healthier" vitamin D levels (Llewellyn et al., 2011; Annweiler and Beauchet, 2014). Recently, a similar study with non-demented German older adults also found that the lowest 25(OH)D quintiles were associated with higher cognitive decline (based on a Cognitive Telephone screening instrument score—COGTEL) over an average 5-year follow-up time (Perna et al., 2014). Interestingly, one study reported that this "positive" effect of 25(OH)D concentrations and cognition tests were only present in the sub-group with low number of years of formal education (Assmann et al., 2015). In the current study, despite the overall low level of standard school education years, no such relationship was observed. Further studies are required to clarify whether there is a minimal 25(OH)D threshold that provides neuroprotection in still cognitive healthy populations.

Our multivariate analysis reveals no effect of 25(OH)D on the temporal trends observed in the MEM and EXEC composites in both groups. With this regard, it is important to consider that in contrast to most studies reporting association with single neuropsychological tests, here multiple cognitive components were characterized and grouped into a comprehensive neuropsychological domain composite. When considered alone, the various studies performed to date were seldom concordant. Two recent meta-analyses, including 14 longitudinal studies, indicate that lower 25(OH)D levels are indeed associated with some executive dysfunctions (especially on mental shifting, information updating and processing speed) and cognitive decline. However, they do not provide clear evidence for the relation of 25(OH)D levels with episodic memory or whether there is a 25(OH)D threshold/optimal therapeutic window for supplementation to prevent cognitive decline later in life (Annweiler et al., 2013; Goodwill and Szoeke, 2017). These same inconsistencies have been reported more recently in larger and longer prospective cohorts. In a large study spanning over 20-years, 25(OH)D levels measured in midlife were not associated with more rapid cognitive decline over the follow-up period (Schneider et al., 2018). However, another large US-based cohort submitted to extensive neuro-cognitive tests covering several domains found that after a mean 5-year follow-up, higher baseline serum 25(OH)D concentrations were linked to a slower rate of decline in a test of verbal fluency (Beydoun et al., 2018). Moreover, a very recent study failed to observe any relationship between baseline 25(OH)D levels and cognition or cognitive decline over 2 years of follow-up by evaluating 1499 Puerto Rican participants living in the Boston Area (aged 45–75 years old at baseline) and using a principal-components analysis to quantify the association between 25(OH)D and the longitudinal performance of two major cognitive features: executive function, and memory (Palacios et al., 2019).

Unfortunately, current published intervention trials addressing vitamin D and cognition have not provided a conclusive answer (Dhesi et al., 2004; Przybelski et al., 2008; Dean et al., 2011; Stein et al., 2011; Rossom et al., 2012; Pettersen, 2017; Rutjes et al., 2018; SanMartin et al., 2018; Castle et al., 2019). A recent small intervention trial showed general cognitive status improvement in 16 mild cognitive impaired patients after 18 months of vitamin D supplementation (but not in healthy controls or those already diagnosed with dementia; SanMartin et al., 2018). Improved visual memory was observed in healthy Canadian adults with "insufficient" baseline 25(OH)D levels (below 75 nmol/l) that were supplemented with high doses of vitamin D (4,000 IU per day) for 18 weeks, without replication in other cognitive domains (Pettersen, 2017). More recently, in a randomized controlled trial with 69 overweight/obese postmenopausal women with 25(OH)D levels less than 75 nmol/l and divided in three different intervention doses of vitamin D (600, 2,000 or 4,000 IU per day), only the intermediate group performed better in learning and memory tests after 1 year supplementation (Castle et al., 2019). These results reflect the complexity behind any potential vitamin D effect on cognition and the need for better-designed studies, probably including distinct vitamin D insufficiency status populations, supplement doses, exposures periods, age groups and baseline cognitive performances.

Another important observation of our study pertains with the 25(OH)D status of the individuals in the study population. Levels below 30 nmol/l were found in 16% and below 50 nmol/l in almost 60%. These results provide a less dramatic indication of vitamin D deficiency/inadequacy compared to recently published data on the Portuguese population (levels below 30/50 nmol/l

were detected in 40/69% of a nationwide cluster sample of 1,500 Portuguese subjects over 65 years old or 38/86% of a subsample of another national cross-sectional study that included Portuguese adults registered in mainland primary health care centers; Raposo et al., 2017; Santos et al., 2017). Much of the differences reported in 25(OH)D levels are probably based on the season-geography confounder and on methodological procedures used (Cashman et al., 2016). In the present study, the LC-MS/MS was used, which provides a more accurate measure of vitamin D status when comparing to some immunological methods used in routine determinations, and this technicality is likely to justify the main differences found between this and other studies coming from similar Portuguese populations (Wallace et al., 2010).

Self-reported daily intake of vitamin D data deserves consideration. Despite the limitation of self-reported data, the estimated median vitamin D intake was only of 0.9 μg/day (36 IU/day; IQR 0.2–3.1 μg/day, 8–124 IU/day), with all subjects evaluated failing to achieve the recommended vitamin D intake (15 μg/day for adults with less than 70 years and 20 μg/day for those older than 70 years; Aloia, 2011). Main dietary sources of vitamin D are meat, fish, milk and cheese. Overall, in our cohort, meat consumption (meat/fish or eggs was present in 54% of the meals with no individuals reporting being vegetarian—data not shown) was relatively small (mean of 145 mg per meal—data not shown). The current Portuguese Food Composition Database states that eggs and meat have generally low levels of vitamin D (between 0.7–1.7 μg per 100 mg; INRJ, 2018). Vitamin D supplementation in food is not mandatory in Portugal and most "supplemented" dairy products present levels of vitamin D between 0.3 and 1.5 μg per 100 ml or 100 g (Parreira et al., 2015). These two facts may imply that, in our sample, even an "usual" daily consumption of 200 g of meat/eggs or "supplemented" dairy products, would represent no more than 20% of the recommended intake of vitamin D. Current habitual intakes of vitamin D in most other countries are also low (typically around 1–4 μg/day), meaning that the proposed reference intake value has to be covered mostly by additional vitamin D supplements and/or endogenous synthesis (Brown et al., 2013). This observation suggests that circulating vitamin D levels in these individuals is most likely provided by exposure to sun. It is important to recall that the present study focuses on a population of active healthy individuals living in the community and that despite the tendency observed towards higher vitamin D intake in the "Good" performing group, no such reflection was present in their circulating 25(OH)D levels.

The study main strengths are grounded on the exhaustive cognitive evaluation performed longitudinally with the construction of coherent executive and memory cognitive domain composites and a two-point season-opposite 25(OH)D levels determination. However, some important limitations of the study should be considered. First, there was a relatively small number of participants available for final exploration with a complete longitudinal cognitive assessment, which decreased the sensitivity to detect any small effect and prevented further analysis comparing distinct groups based on more extreme 25(OH)D distribution. Second, there was a relatively short time between the two cognitive evaluations (min-max: 16–22 months), which may have also impaired the ability to detect subtle intra or inter-individual cognitive time-dependent differences, underestimating potential associations with 25(OH)D levels. Third, despite the two seasonal opposite 25(OH)D determinations separated by at least 1.5-years, no "real" prolonged longitudinal evaluation of 25(OH)D levels was made. This means that individual vitamin D status appraisal may have resulted in a rather 2-year "cross-sectional" estimation and prevented, therefore, any assessment of its expanded longitudinal effect on cognition. Also relevant is the fact that the study population was almost homogenously vitamin D insufficient. This feature, frequently found in other studies with older adults, makes it harder to discover any associations given the lack of 25(OH)D levels inter-individual variability found in these cohorts (Annweiler et al., 2011).

In summary, in this Portuguese older healthy population, serum 25(OH)D levels were not associated with cognitive domain composite scores for an average 18 months follow-up, in both stronger and poorer cognitive performers. Despite the lack of agreement about what should be the optimal circulating 25(OH)D levels, the conclusions of the present study are limited to the neurocognitive outcomes obtained in older individuals with relatively stable low 25(OH)D levels and during a short longitudinal period. Further studies should address the role of vitamin D during several brain development time frames and its role in cognitive aging.

ETHICS STATEMENT

This study was performed in accordance with the Declaration of Helsinki and approved by national and local ethics review boards (Hospital de Braga, Centro Hospitalar do Alto Ave and Unidade Local de Saúde do Alto Minho) and by the national data protection entity (Comissão Nacional de Protecção de Dados). All study goals and nature of the tests were explained to the potential participants and informed signed consent obtained.

AUTHOR CONTRIBUTIONS

AC, NCS, NS and JP designed the study protocol. AC, NCS, CP-N, TC and PM collected the data. Data analysis and interpretation by AC, NCS and PC. AC wrote the first draft under supervision of JP. AC, NCS, CP-N, TC, PM, PC, NS and JP contributed significantly to revising the manuscript. All authors read and approved the final manuscript.

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REFERENCES

Aloia, J. F. (2011). Clinical review: the 2011 report on dietary reference intake for vitamin D: where do we go from here? *J. Clin. Endocrinol. Metab.* 96, 2987–2996. doi: 10.1210/jc.2011-0090

Anastasiou, C. A., Yannakoulia, M., and Scarmeas, N. (2014). Vitamin D and cognition: an update of the current evidence. *J. Alzheimers Dis.* 42, S71–S80. doi: 10.3233/JAD-132636

Annweiler, C., and Beauchet, O. (2014). Vitamin d in older adults: the need to specify standard values with respect to cognition. *Front. Aging Neurosci.* 6:72. doi: 10.3389/fnagi.2014.00072

Annweiler, C., Montero-Odasso, M., Llewellyn, D. J., Richard-Devantoy, S., Duque, G., and Beauchet, O. (2013). Meta-analysis of memory and executive dysfunctions in relation to vitamin D. *J. Alzheimers Dis.* 37, 147–171. doi: 10.3233/JAD-130452

Annweiler, C., Schott, A. M., Allali, G., Bridenbaugh, S. A., Kressig, R. W., Allain, P., et al. (2010). Association of vitamin D deficiency with cognitive impairment in older women: cross-sectional study. *Neurology* 74, 27–32. doi: 10.1212/WNL.0b013e3181beecd3

Annweiler, C., Souberbielle, J. C., Schott, A. M., De Decker, L., Berrut, G., and Beauchet, O. (2011). Vitamin D in the elderly: 5 points to remember. *Geriatr. Psychol. Neuropsychiatr. Vieil.* 9, 259–267. doi: 10.1684/pnv.2011.0288

Assmann, K. E., Touvier, M., Andreeva, V. A., Deschasaux, M., Constans, T., Hercberg, S., et al. (2015). Midlife plasma vitamin D concentrations and performance in different cognitive domains assessed 13 years later. *Br. J. Nutr.* 113, 1628–1637. doi: 10.1017/s0007114515001051

Aung, K., Burnett, J., Smith, S. M., and Dyer, C. B. (2006). Vitamin D deficiency associated with self-neglect in the elderly. *J. Elder Abuse Negl.* 18, 63–78. doi: 10.1300/j084v18n04_07

Barroso, T. (2014). *Alimentos Enriquecidos com Vitaminas e Minerais [Food Enriched with Vitamins and Minerals]*. Lisbon, Portugal: University of Lisbon. Master Thesis.

Beydoun, M. A., Hossain, S., Fanelli-Kuczmarski, M. T., Beydoun, H. A., Canas, J. A., Evans, M. K., et al. (2018). Vitamin D status and intakes and their association with cognitive trajectory in a longitudinal study of urban adults. *J. Clin. Endocrinol. Metab.* 103, 1654–1668. doi: 10.1210/jc.2017-02462

Bouillon, R. (2016). "Chapter 59–Vitamin D: from photosynthesis, metabolism and action to clinical applications* A2-Jameson, J. Larry," in *Endocrinology: Adult and Pediatric (Seventh Edition)*, eds L. J. D. Groot, D. M. D. Kretser, L. C. Giudice, A. B. Grossman, S. Melmed, J. T. Potts and G. C. Weir (Philadelphia, PA: W.B. Saunders), 1018.e1017–1037.e1017.

Brown, J., Ignatius, A., Amling, M., and Barvencik, F. (2013). New perspectives on vitamin D sources in Germany based on a novel mathematical bottom-up model of 25(OH)D serum concentrations. *Eur. J. Nutr.* 52, 1733–1742. doi: 10.1007/s00394-012-0477-3

Busch, R. M., and Chapin, J. S. (2008). Review of normative data for common screening measures used to evaluate cognitive functioning in elderly individuals. *Clin. Neuropsychol.* 22, 620–650. doi: 10.1080/13854040701448793

Cashman, K. D., Dowling, K. G., Skrabakova, Z., Gonzalez-Gross, M., Valtuena, J., De Henauw, S., et al. (2016). Vitamin D deficiency in Europe: pandemic? *Am. J. Clin. Nutr.* 103, 1033–1044. doi: 10.3945/ajcn.115.120873

Castle, M., Fiedler, N., Pop, L. C., Schneider, S. J., Schlussel, Y., Sukumar, D., et al. (2019). Three doses of vitamin D and cognitive outcomes in older women: a double-blind randomized controlled trial. *J. Gerontol. A Biol. Sci. Med. Sci.* doi: 10.1093/gerona/glz041 [Epub ahead of print].

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences (2nd Edn.)*. Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers.

Costa, P. S., Santos, N. C., Cunha, P., Palha, J. A., and Sousa, N. (2013). The use of bayesian latent class cluster models to classify patterns of cognitive performance in healthy ageing. *PLoS One* 8:e71940. doi: 10.1371/journal.pone.0071940

Dean, A. J., Bellgrove, M. A., Hall, T., Phan, W. M., Eyles, D. W., Kvaskoff, D., et al. (2011). Effects of vitamin D supplementation on cognitive and emotional functioning in young adults-a randomised controlled trial. *PLoS One* 6:e25966. doi: 10.1371/journal.pone.0025966

Dhesi, J. K., Jackson, S. H., Bearne, L. M., Moniz, C., Hurley, M. V., Swift, C. G., et al. (2004). Vitamin D supplementation improves neuromuscular function in older people who fall. *Age Ageing* 33, 589–595. doi: 10.1093/ageing/afh209

Goodwill, A. M., Campbell, S., Simpson, S. Jr., Bisignano, M., Chiang, C., Dennerstein, L., et al. (2018). Vitamin D status is associated with executive function a decade later: data from the women's healthy ageing project. *Maturitas* 107, 56–62. doi: 10.1016/j.maturitas.2017.10.005

Goodwill, A. M., and Szoeke, C. (2017). A systematic review and meta-analysis of the effect of low vitamin D on cognition. *J. Am. Geriatr. Soc.* 65, 2161–2168. doi: 10.1111/jgs.15012

Granic, A., Aspray, T., Hill, T., Davies, K., Collerton, J., Martin-Ruiz, C., et al. (2015). 25-hydroxyvitamin D and increased all-cause mortality in very old women: the Newcastle 85+ study. *J. Intern. Med.* 277, 456–467. doi: 10.1111/joim.12273

Grigoletto, F., Zappalà, G., Anderson, D. W., and Lebowitz, B. D. (1999). Norms for the mini-mental state examination in a healthy population. *Neurology* 53, 315–320. doi: 10.1212/wnl.53.2.315

Guerreiro, M., Silva, A. P., Botelho, M. A., Leitão, O., Castro-Caldas, A., and Garcia, C. (1994). Adaptação à população portuguesa da tradução do Mini Mental State Examination (MMSE). *Rev. Port. Neurol.* 1, 9–10.

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., et al. (2011). Evaluation, treatment and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J. Clin. Endocrinol. Metab.* 96, 1911–1930. doi: 10.1210/jc.2011-0385

INRJ. (2018). "Portuguese food compositon database," in *Centro de Segurança Alimentar e Nutrição, Instituto Nacional Dr. Ricardo Jorge*. Lisbon: Portuguese Ministry of Health. Available online at: http://portfir.insa.pt/.

Kuźma, E., Soni, M., Littlejohns, T. J., Ranson, J. M., Van Schoor, N. M., Deeg, D. J., et al. (2016). Vitamin D and memory decline: two population-based prospective studies. *J. Alzheimers Dis.* 50, 1099–1108. doi: 10.3233/JAD-150811

Lim, A. S. P., Gaiteri, C., Yu, L., Sohail, S., Swardfager, W., Tasaki, S., et al. (2018). Seasonal plasticity of cognition and related biological measures in adults with and without Alzheimer disease: analysis of multiple cohorts. *PLoS Med* 15:e1002647. doi: 10.1371/journal.pmed.1002647

Llewellyn, D. J., Lang, I. A., Langa, K. M., and Melzer, D. (2011). Vitamin D and cognitive impairment in the elderly U.S. population. *J. Gerontol. A Biol. Sci. Med. Sci.* 66, 59–65. doi: 10.1093/gerona/glq185

Meyer, C., Muto, V., Jaspar, M., Kusse, C., Lambot, E., Chellappa, S. L., et al. (2016). Seasonality in human cognitive brain responses. *Proc. Natl. Acad. Sci. U S A* 113, 3066–3071. doi: 10.1073/pnas.1518129113

Moreira, P. S., Santos, N., Castanho, T., Amorim, L., Portugal-Nunes, C., Sousa, N., et al. (2018). Longitudinal measurement invariance of memory performance and executive functioning in healthy aging. *PLoS One* 13:e0204012. doi: 10.1371/journal.pone.0204012

Palacios, N., Scott, T., Sahasrabudhe, N., Gao, X., and Tucker, K. L. (2019). Serum vitamin D and cognition in a cohort of Boston-area Puerto Ricans. *Nutr. Neurosci.* doi: 10.1080/1028415x.2018.1545291 [Epub ahead of print].

Parreira, D., Serra, M. C., and Dias, M. G. (2015). Vitamin D levels in products dairy products consumed in Portugal. *Bol. Epidemiologico Inst. Nac. Ricardo Jorge* 12, 27–28.

Perna, L., Mons, U., Kliegel, M., and Brenner, H. (2014). Serum 25-hydroxyvitamin D and cognitive decline: a longitudinal study among non-demented older adults. *Dement. Geriatr. Cogn. Disord.* 38, 254–263. doi: 10.1159/000362870

Pettersen, J. A. (2017). Does high dose vitamin D supplementation enhance cognition?: a randomized trial in healthy adults. *Exp. Gerontol.* 90, 90–97. doi: 10.1016/j.exger.2017.01.019

Pludowski, P., Holick, M. F., Pilz, S., Wagner, C. L., Hollis, B. W., Grant, W. B., et al. (2013). Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-a review of recent evidence. *Autoimmun. Rev.* 12, 976–989. doi: 10.1016/j.autrev.2013.02.004

Przybelski, R., Agrawal, S., Krueger, D., Engelke, J. A., Walbrun, F., and Binkley, N. (2008). Rapid correction of low vitamin D status in nursing home residents. *Osteoporos. Int.* 19, 1621–1628. doi: 10.1007/s00198-008-0619-x

Raposo, L., Martins, S., Ferreira, D., Guimaraes, J. T., and Santos, A. C. (2017). Vitamin D, parathyroid hormone and metabolic syndrome-the PORMETS study. *BMC Endocr. Disord.* 17:71. doi: 10.1186/s12902-017-0221-3

Rossom, R. C., Espeland, M. A., Manson, J. E., Dysken, M. W., Johnson, K. C., Lane, D. S., et al. (2012). Calcium and vitamin D supplementation and cognitive impairment in the women's health initiative. *J. Am. Geriatr. Soc.* 60, 2197–2205. doi: 10.1111/jgs.12032

Rutjes, A. W., Denton, D. A., Di Nisio, M., Chong, L. Y., Abraham, R. P., Al-Assaf, A. S., et al. (2018). Vitamin and mineral supplementation for maintaining cognitive function in cognitively healthy people in mid and late life. *Cochrane Database Syst. Rev.* 12:CD011906. doi: 10.1002/14651858.CD011906.pub2

SanMartin, C. D., Henriquez, M., Chacon, C., Ponce, D. P., Salech, F., Rogers, N. K., et al. (2018). Vitamin D increases Aβ140 plasma levels and protects lymphocytes from oxidative death in mild cognitive impairment patients. *Curr. Alzheimer Res.* 15, 561–569. doi: 10.2174/1567205015666171227154636

Santos, A., Amaral, T. F., Guerra, R. S., Sousa, A. S., Alvares, L., Moreira, P., et al. (2017). Vitamin D status and associated factors among portuguese older adults: results from the nutrition UP 65 cross-sectional study. *BMJ Open* 7:e016123. doi: 10.1136/bmjopen-2017-016123

Santos, N. C., Costa, P. S., Cunha, P., Cotter, J., Sampaio, A., Zihl, J., et al. (2013). Mood is a key determinant of cognitive performance in community-dwelling older adults: a cross-sectional analysis. *Age* 35, 1983–1993. doi: 10.1007/s11357-012-9482-y

Santos, N. C., Costa, P. S., Cunha, P., Portugal-Nunes, C., Amorim, L., Cotter, J., et al. (2014). Clinical, physical and lifestyle variables and relationship with cognition and mood in aging: a cross-sectional analysis of distinct educational groups. *Front. Aging Neurosci.* 6:21. doi: 10.3389/fnagi.2014.00021

Schneider, A. L. C., Zhao, D., Lutsey, P. L., Gottesman, R. F., Sharrett, A. R., Rawlings, A. M., et al. (2018). Serum vitamin D concentrations and cognitive change over 20 years: the atherosclerosis risk in communities neurocognitive study. *Neuroepidemiology* 51, 131–137. doi: 10.1159/000490912

Schöttker, B., Jorde, R., Peasey, A., Thorand, B., Jansen, E. H. J. M., Groot, L. D., et al. (2014). Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. *BMJ* 348:g3656. doi: 10.1136/bmj.g3656

Stein, M. S., Scherer, S. C., Ladd, K. S., and Harrison, L. C. (2011). A randomized controlled trial of high-dose vitamin D2 followed by intranasal insulin in Alzheimer's disease. *J. Alzheimers Dis.* 26, 477–484. doi: 10.3233/JAD-2011-110149

van der Schaft, J., Koek, H. L., Dijkstra, E., Verhaar, H. J., Van Der Schouw, Y. T., and Emmelot-Vonk, M. H. (2013). The association between vitamin D and cognition: a systematic review. *Ageing Res. Rev.* 12, 1013–1023. doi: 10.1016/j.arr.2013.05.004

Wallace, A. M., Gibson, S., De La Hunty, A., Lamberg-Allardt, C., and Ashwell, M. (2010). Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations. *Steroids* 75, 477–488. doi: 10.1016/j.steroids.2010.02.012

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