Universidade do Minho
Escola de Medicina

José Carlos Leitão Portugal Nunes

Exploring the crosstalk between mood and metabolism: new insights from ageing, obesity and depression

fevereiro de 2020

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UMinho | 2020

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Tese de Doutoramento
Programa Interuniversitário de Doutoramento em Envelhecimento e Doenças Crónicas

Trabalho efetuado sob a orientação do
Professor Doutor João Miguel Bessa
e da
Professora Doutora Maria Paula Macedo

fevereiro de 2020

ACKNOWLEDGMENTS

The first acknowledge goes to my Supervisor Professor João Bessa. I am not able to express in words all the gratitude that you deserve. For all the patience that you have and for the calming words that you said in the moments of my despair. Thank you for your friendship also. I also want to acknowledge Professor Paula Macedo, also my Supervisor, for being so kind and available. I am only able to finish this journey because someone gave me the chance to start it, and for that I acknowledge to Professor Joana Palha.

To the most important person in my life, Iris. It was a long and windy journey and I am sure that I only reached the end because I have you by my side. You make me the best I can be, and you bring to me the best a life can give. I hope I deserve you and give it back to you.

To my family, friends and colleagues that I cannot mention due to space limitations.

The work presented in this thesis was performed in the Life and Health Sciences Research Institute (ICVS), Minho University and the Clinical Academic Center (2CA), Hospital of Braga. Financial support was provided by grants from the Foundation for Science and Technology (FCT) through the PhD grant in the Inter-University Doctoral Programme in Aging and Chronic Disease (PhDOC) with the reference PD/BD/106050/2015, by FEDER funds through the Operational Programme Competitiveness Factors - COMPETE and National Funds through FCT - Foundation for Science and Technology under the project PTDC/DTP-PIC/6936/2014; and by the projects NORTE-01-0145-FEDER-000013 and NORTE-01-0145-FEDER-000023, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (FEDER). Financial Support was also obtained from "SwitchBox" (Contract HEALTH-F2-2010-259772), co-financed by the Portuguese North Regional Operational Program (ON.2 – O Novo Norte), under the National Strategic Reference Framework (QREN), through the European Regional Development Fund (FEDER), and by the Fundação Calouste Gulbenkian (Portugal) (Contract grant number: P-139977; project "Better mental health during ageing based on temporal prediction of individual brain ageing trajectories (TEMPO)").

STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

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

Explorar o diálogo entre o humor e o metabolismo: novas perspetivas do envelhecimento, obesidade e depressão.

RESUMO

Ao longo dos anos, vários estudos mostraram que o humor depressivo e a depressão estão associados à obesidade, resistência à insulina, diabetes e síndrome metabólica. A existência dessas associações aumenta a plausibilidade de um vínculo biológico entre essas condições, mas os mecanismos envolvidos na associação de humor e distúrbios metabólicos permanecem obscuros.

Em indivíduos idosos, a tendência da associação entre obesidade e humor é controversa; portanto, exploramos o impacto da idade na associação entre humor depressivo e índices de adiposidade. Observamos que a idade é um moderador relevante na associação entre humor depressivo e adiposidade em idosos. Além disso, a análise diferencial dos compartimentos de gordura corporal revelou que a associação entre o tecido adiposo visceral e o humor depressivo é positiva independentemente da idade. Como o tecido adiposo visceral está associado a complicações metabólicas e a síndrome metabólica também tem sido associada ao humor depressivo, exploramos ainda essa associação. Observamos que a disfunção metabólica está associada ao humor depressivo, e alguns componentes dos seus componentes estão associados de maneira dependente da idade. Além disso, a interação do humor com os componentes da disfunção metabólica é refletida no nível central em padrões diferenciais de conectividade funcional da rede neuronal conhecida por *default mode network*.

A cirurgia bariátrica oferece uma oportunidade relevante de estudar a associação entre humor e cognição. Para melhor compreender essa associação exploramos a dinâmica temporal da relação entre biomarcadores metabólicos e desempenho neuro-cognitivo após cirurgia bariátrica. Observamos que os biomarcadores metabólicos estão correlacionados com ansiedade, humor depressivo e desempenho cognitivo em pacientes submetidos a cirurgia bariátrica. Essas associações não são estáveis, mas evoluem à medida que o intervalo após a cirurgia aumenta.

Também exploramos os correlatos neurais da depressão e do tratamento antidepressivo com paroxetina no processamento neural da informação associada ao apetite e na composição corporal. Pacientes deprimidos apresentam uma diminuição da ativação cerebral em áreas associadas ao processamento de estímulos alimentares e áreas que integram respostas somáticas aos alimentos. Áreas associadas à recompensa, tomada de decisão e integração de estímulos sensoriais apresentam maior ativação após 6 a 8 semanas de tratamento com paroxetina.

Palavras-chave: cognição, envelhecimento, humor, metabolismo, obesidade

Exploring the crosstalk between mood and metabolism: new insights from ageing, obesity and depression.

ABSTRACT

Over the years several studies have shown that depressive mood and depression are associated with obesity, insulin resistance, diabetes and metabolic syndrome. The existence of these associations increases the plausibility of a biological link between those conditions, yet the mechanisms and pathways for the association of mood and metabolic disturbance remains unclear.

In older individuals the trend of the association between obesity and mood is controversial; therefore, we explored the impact of age upon the association between depressive mood and adiposity indexes. We observe that age is a relevant moderator in the association between depressive mood and adiposity in the elderlies. Furthermore, the body fat compartment analysis revealed that the association of visceral adipose tissue and depressive mood is positive independently of age.

Since visceral adipose tissue is associated with metabolic complications and metabolic syndrome has also been associated with depressive mood we further explored those associations. Metabolic dysfunction is associated with depressive mood, and some components of metabolic dysfunction are associated in an age dependent manner. Furthermore, the interaction of mood and components of metabolic dysfunction is reflected at the central level in differential patterns of default mode network functional connectivity.

Bariatric surgery offers an important way to study the association between mood and cognition. We explored the temporal dynamics of the relationship between metabolic biomarkers and neurocognitive performance after bariatric surgery. Metabolic biomarkers are correlated with anxiety, depressive mood and cognitive performance in bariatric surgery patients. These associations are not stable but rather evolve as the interval following surgery increased.

We also explored the neural correlates of depression and antidepressant treatment with paroxetine in neural processing of appetite information and in body composition. Depressed patients present a decrease brain activation in areas associated with the processing of food stimuli and areas that integrate somatic responses to food. Areas associated with reward, decision-making and integration of sensory cues present a higher activation after 6 to 8 weeks of treatment with paroxetine.

Key Words: aging, cognition metabolism, mood, obesity,

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LIST OF ABBREVIATIONS

2CA-B – Clinical Academic Center – Braga

A

AAL – anatomical automatic labeling

ACTH – adrenocorticotropin

B

BDNF – brain-derived neurotrophic factor

BF – body fat

BMI – body mass index

BOLD – blood oxygen level dependent

BP – blood pressure

C

C – colors

CFA – confirmatory factor analysis

CFI – comparative fit index

CLTR – consistent long-term retrieval

CRH – corticotropin-releasing hormone

CSF – cerebrospinal fluid

CW – colors/words

D

DMN – default mode network

DMN-FC – default mode network functional connectivity

DR – delayed recall

DSM-5 – 5th edition of the Diagnostic and Statistical Manual of Mental Disorders

E

EPI – gradient echo-weighted echo-planar images

EWL – excessive e weight loss

F

FA – flip angle

FC – functional connectivity

FCT – Portuguese Foundation for Science and Technology

fMRI – functional magnetic resonance image

FOV – field of view

FSL – FMRIB Software Library

G

GDS – geriatric depression scale

GEE – generalized estimating equations

GIP – gastric inhibitory polypeptide

GLM – general linear model

GLP-1 – glucagon-like peptide-1

H

HADS – hospital anxiety and depression scale

HAM-A – Hamilton anxiety rating scale

HAM-D – Hamilton depression rating scale

HDL – high-density lipoprotein

HOMA2-IR – homeostatic model assessment indexes of insulin resistance

HPA – hypothalamus-pituitary-adrenal

I

IAN-AF – national food, nutrition and physical activity survey

IAPS – international affective picture system

ICD-11 – 11th revision of the international classification of diseases and related health problems

ICVS – life and health sciences research institute

IDF – international diabetes federation

IFSO – international federation for the surgery of obesity and metabolic disorders

IL – interleukin

IQR – inter-quartile range

L

LABS – longitudinal assessment of bariatric surgery

LDL – low-density lipoprotein

LTS – long term storage

M

M1 – moment 1 (baseline)

M2 – moment 2 (reassessment)

M – mean

Md – median

MDD – major depressive disorder

MELODIC - multivariate exploratory linear optimized decomposition into independent components

MetS – metabolic syndrome

MLR – maximum likelihood parameter estimates with standard errors and a chi-square test statistic that are robust to non-normality and non-independence of observations

MLRM – multiple linear regression models

MNI - Montreal neurological institute

MPRAGE – magnetization-prepared rapid acquisition by gradient echo

MRI – magnetic resonance image

N

NCEP-ATP III – national cholesterol education program - adult treatment panel-iii

NGF – nerve growth factor

NIRKO – Brain-specific knockout of insulin receptor

NT – neurotrophin

O

OLAF - open library of affective foods

P

PAI-1 – plasminogen activator inhibitor-1

PICA – probabilistic independent component analysis

PSS – perceived stress scale

R

RMSEA – root mean square error of approximation

ROI – region of interest

RSN – resting state network

RYGB - roux-en-Y gastric by-pass

S

SAT – subcutaneous adipose tissue

SD – standard deviation

SEM – structural equation models

SG – sleeve gastrectomy

SPM – statistical parametric mapping

SRT – selective reminding test

SSRI – selective serotonin reuptake inhibitor

T

TE – echo time

TFEQ – three-factor eating questionnaire

TLI – Tucker-Lewis index

TNF – tumor necrosis factor

TR – repetition time

TRUFI – true fast imaging with steady state precession images

V

VAT – visceral adipose tissue

W

W – words

WC – waist circumference

WHO – world health organization

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CHAPTER I

Introduction

INTRODUCTION

Over the years several studies have been published addressing the association between mood and metabolic status. Evidence from systematic reviews and meta-analytic studies have shown that depressive mood and depression are associated with obesity [1-5], insulin resistance [6], diabetes [7-9] and metabolic syndrome [10-13]. The existence of these associations increases the plausibility of a biological link between those conditions, yet the mechanisms and pathways for the association of mood and metabolic disturbance remains unclear. Understanding these relationships can open new routes to target modifiable factors that can influence both mood and metabolic disturbance.

In this chapter we will define the concepts under analysis, address the relevance of understanding the association between mood and metabolism and the potential involved mechanisms.

MAJOR DEPRESSIVE DISORDER AND DEPRESSIVE MOOD

Overview

Dominant systems for diagnostic and classification for depression are based on a binary approach [14-16]. According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [17] the diagnosis of major depressive disorder (MDD) is made in the continuous presence, for at least 2 weeks, of 5 or more of the following symptoms: must include at least one of (I) depressed mood (subjective or observed);and (II) loss of interest or pleasure; plus, (III) change in weight or appetite; (IV) insomnia or hypersomnia; (V) psychomotor retardation or agitation (observed); (VI) loss of energy or fatigue; (VII) worthlessness or guilt; (VIII) impaired concentration or indecisiveness; and (IX) thoughts of death or suicidal ideation or attempt. Consistent with DSM-5, according to the 11th revision of the International Classification of Diseases and Related Health Problems (ICD-11) for the diagnostic guidelines for depressive disorder a minimum of five of ten symptoms is required [18]. A depressive disorder is characterized by a period of almost daily (I) depressed mood or (II) diminished interest in activities lasting at least two weeks accompanied by other symptoms such as (III) difficulty concentrating, (IV) feelings of worthlessness or excessive or inappropriate guilt, (V) hopelessness, (VI) recurrent thoughts of death or suicide, (VII) changes in appetite or (VII) sleep, (IX) psychomotor agitation or retardation, and (X) reduced

energy or fatigue [19]. Importantly, in ICD-11 the temporal pattern is used as a basis for determining which mood disorder best fits the clinical presentation (for instance single episode depressive disorder or recurrent depressive disorder) [18,19].

Despite the binary classification of depression, most research points to the continuous and dimensional nature of this disorder [14,20], meaning that there are individuals who have a diagnosable disorder according the criteria mentioned, others have depressive symptomatology that can be considered subsyndromal, and do not meet the diagnostic criteria for depressive disorders, but are also in distress, and should therefore benefit from intervention. In the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) the clinical significance of some subthreshold forms of depression, such as minor depression was acknowledged [21]. Depressive mood and symptomatology have been found to increase the risk of developing major depression, considerably affect quality of life, increase health service utilization and to cause economic damage because of the associated disability days [22]. The high prevalence, and the associated psychosocial impairment, make depressive mood and symptomatology a matter for consideration by clinicians and researchers [21].

Epidemiology

Worldwide, depression is the leading cause of mental health-related disease burden, affecting an estimated 300 million people [14]. Considering The Global Burden of Diseases, Injuries, and Risk Factors Study 2017 [23] depressive disorders (major depressive disorder and dysthymia) are the third leading cause of burden of disease among the conditions that were studied. Depressive disorders represent a barrier to sustainable development by preventing individuals from reaching their full potential, impairs human capital, and is associated with premature mortality [14]. In Portugal, along with North Ireland, the prevalence of mental disorders is one of the highest in Europe, with depressive disorders being the second most prevalent [24].

Pathophysiology

The pathophysiology of MDD is unclear and remains elusive [25]. As previously mentioned, contrary to other diseases, diagnosis of depression is not based on objective criteria but in variable set of symptoms; therefore, depression might be seen as a heterogeneous syndrome composed by numerous diseases

that may have distinct causes and origins [26]. Depression may also be caused by an matrix of reciprocally interactive pathophysiological mechanisms in which all the mechanisms are related and interact bidirectionally [27].

Several hypotheses have been proposed to explain the etiology of depression. During many years the hypothesis of monoamine depletion was used to explain the pathophysiology of depression. Other theories that have been proposed to explain the underlying biological mechanisms of depression include the neuroendocrine, neurotrophic and neuroimmune hypothesis [25,26].

The monoamine hypothesis was derived from the clinical finding that monoamine depletion by reserpine, an antihypertensive drug, caused depression in patients not suffering of the disease before reserpine therapy [28]. This hypothesis was supported by the serendipitous discovery that tricyclic agents and monoamine oxidase inhibitors cause the short-term increase the concentration of monoamines in synapses [25,28]. The tricyclic agents evolved from antihistamine research and monoamine oxidase inhibitors were originated from work on antitubercular drugs [26]. Brain neurochemistry was one of the first aspects that was examined in the study of the molecular basis of depression and the discovery that depression could be treated or triggered with those medications provided one of the first clues into the types of chemical changes in the brain that regulate depressive symptoms [26,28].

Other theory for the pathophysiology of depression is the neuroendocrine theory. This theory expresses that the dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis is involved in the in the pathophysiology of depression [25]. Corticotropin-releasing factor is secreted by neurons in the paraventricular nucleus of the hypothalamus and stimulates the synthesis and release of adrenocorticotropin from the anterior pituitary. Synthesis and release of glucocorticoids (cortisol in humans) from the adrenal cortex is then stimulated by adrenocorticotropin. Glucocorticoids exert profound effects on general metabolism and affect behavior by acting on several brain regions. The sustained elevation of glucocorticoids, that is seen under conditions of prolonged and severe stress, damage hippocampal CA3 pyramidal neurons. Stress and the resulting hypercortisolemia reduce the birth of new neurons in the adult hippocampal dentate gyrus and is involved in the reduction of dendritic branching and a loss of dendritic spines where the neurons receive their inputs [26,29]. Many depressed patients present increased size and activity of the pituitary and adrenal glands, as well as increased levels of cortisol in the saliva, plasma and urine. Glucocorticoids are important regulators of neuronal survival, neurogenesis, size of complex anatomical structures such as the hippocampus, acquisition of new

memories and the emotional appraisal of events. Therefore, it is not surprising that the HPA axis has been found to be abnormal in depression considering its role at the interface between stress and brain functioning [29]. Further supporting the involvement of the HPA axis in the pathophysiology of depression antidepressants are able to downregulate the HPA axis hyperactivity [25,30].

The neuroendocrine theory is not limited to the role of the HPA axis in depression. Impaired leptin production or leptin resistance and insulin resistance have been also associated with depression [31,32]. Several lines of evidence indicate that leptin has neurotrophic effects and that brain areas implicated in the control of mood and emotion, such as the hippocampus, cortex, and amygdala, present a high expression of leptin receptors. Studies on animal models with mutations in the leptin gene or the leptin receptor gene have provided information on the relationship between leptin and depression. Both alterations lead to reduced brain weight and cortical volume and decreased expression of total neuronal and glial proteins [32]. In humans, controversial results have been observed regarding leptin levels and depression. Studies have shown no difference in leptin levels between depressed patients and controls [33], some have shown higher levels of plasma leptin in depressed patients [34-37], and others demonstrated that plasma leptin levels were decreased in patients with major depression [38,39]. Furthermore, decreased levels of leptin were found in cerebrospinal fluid (CSF) of suicide attempters [40,41] and in plasma of patients with bipolar disorder [42] and obsessive-compulsive disorder with comorbid major depression [43]. Several factors, including leptin resistance, may explain these contradictory results [32].

Insulin resistance has also been associated with depression. The prevalence of depression is estimated to be three times greater in diabetic patients than in the general population [44]. This association has been extensively reviewed in several systematic reviews and meta-analysis [6-9,45-47]. Those studies consistently indicate a positive association between dysregulation of the glucose homeostasis and depression both in cross-sectional and longitudinal analysis. Studies on animal models and humans with diabetes show that insulin disturbances affect the production and metabolism of brain monoamines and the expression and function of serotoninergic receptors may also be altered [44]. Recently, data from animal models indicate that high fat diet induces brain insulin resistance associated with altered behavior in mice [48]. Furthermore, insulin action has been shown to regulate neuronal signaling and plasticity [49]. Brain-specific knockout of insulin receptor (NIRKO) in mice has been shown to be associated with age-related anxiety and depressive-like behavior, altered mitochondrial function, aberrant monoamine oxidase expression, and increased dopamine turnover in the mesolimbic system [50].

The neuroimmune theory of depression states the involvement of immune mediators in the etiology of depression. The original link between immune mediators and depression was a result of studies that examined psychiatric complications following long term treatment with interferon alpha for hepatitis C [51]. Those immune mediators, such as cytokines like interferons and interleukins (IL), are modulators of important biological functions and alteration of those functions may contribute to the pathogenesis of depression [25]. Data from animal models indicate that cytokines may act on neurons altering plasticity and promoting depression-like behaviors. For instance, intracranial infusions of IL-6 or administration of IL-1b into the hippocampus increase depression associated behavior and reduces neurogenesis, whereas infusion of IL-1b and IL-6 antibodies, or genetic deletion of IL-6, blocks the depressive-like effects induced by CMS. Cytokines also can act on serotonin neurons through the kynurenine pathway and tryptophan catabolites as well as directly on glutamatergic neurons in the frontal cortex and hippocampus to alter synaptic plasticity. They also can activate a microglia-dependent phagocytic process that engulfs and clears excitatory synapses altering synaptic transmission and behavior [51]. Furthermore, several lines of evidence demonstrate that cytokines can contribute to HPA axis hyperactivity and subsequently lead to depressive symptomatology. Pro-inflammatory cytokines can stimulate glucocorticoid release by acting at all levels of the HPA axis:, they stimulate the release of corticotropin-releasing hormone (CRH) at the paraventricular nucleus; they stimulate the release of adrenocorticotropin (ACTH) at the pituitary; and at the adrenal glands they stimulate the release of glucocorticoids [25,51].

Further supporting the role of immune function in depression there are meta-analysis reporting significantly higher concentrations of the proinflammatory cytokines, tumor necrosis factor (TNF)α and IL-6 in depressed subjects compared with control subjects [52]. Also, antidepressant treatment was associated with a decrease in IL-6 levels whereas persistently elevated TNF-α was associated with antidepressant treatment resistance [53].

The neurotrophic theory of depression is the most recent theory that was put forward. This hypothesis implies that neurotrophic factors, neuroplasticity and adult neurogenesis and other related aspects of hippocampal plasticity are involved in the pathophysiology of MDD and its effective treatment [25,54]. Neurotrophins are growth factors with crucial roles in neurogenesis and neuroplasticity. The neurotrophin family include molecules such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4). Further supporting this hypothesis it was shown that depressed patients present decreased serum levels of BDNF and the treatment with antidepressants

promotes an increase in these levels [54]. Animal models submitted to stress protocols, to induce depressive-like behavior, show a decrease in levels of BDNF levels in the hippocampus. Also, antidepressant treatment was able to restore the levels of BDNF in the hippocampus [55].

Many factors may be involved in the etiology of depression. Genetic components may be important risk factors for depression, in fact, epidemiological studies suggest that the contribution of genetic is accountable for 40 to 50% of the risk factors for depression. Since depression is a complex disorder is possible that many genes may be involved which may has been frustrating the finding of the genetic basis of depression. Also non-genetic factors, such as stress, trauma, innumerable medical conditions and even processes during brain development, are important factors in the etiology of depression [26].

Treatment and treatment response

Similar to the etiology of depression, the mechanisms that underly the treatment of depression also remain elusive [56]. Despite not fully understood, there several effective treatment for depression, namely antidepressants drugs, electroconvulsive therapy and several form of psychotherapy [26]. Currently, antidepressant drugs constitute the standard of care for major depression disorder and selective serotonin reuptake inhibitors (SSRI) are often used as the first-line of pharmacotherapy [57]. For the propose of this thesis we will only address antidepressant drugs, particularly SSRIs.

A revolution in the fields of psychopharmacology and psychiatry took place around the 1950s with the clinical introduction of the main groups of psychoactive drugs still used today. The serendipitous discovery that tricyclic agents (imipramine) and monoamine oxidase inhibitors (iproniazid) cause the short-term increase the concentration of monoamines in synapsis [25,28]. The tricyclic agents evolved from antihistamine research and monoamine oxidase inhibitors were originated from work on antitubercular drugs [26]. The discovery of those agents allowed further research and the development of numerous second-generation drugs, such as SSRIs. Fluoxetine, a SSRI, was introduced in the market in 1987, and revolutionized the therapy for depression. SSRIs have a better side effect profile than the older agents to treat depression, such as the lack of anticholinergic and cardiac effects. However, SSRIs are also not devoid of considerable tolerability issues and some patients experience common acute treatment adverse effects such as nausea, insomnia, headaches, dizziness, gastrointestinal symptoms and sexual

dysfunction and long-term adverse effects including weight gain, sexual dysfunction and sleep disturbances [58].

All the available antidepressants continue to employ the same mechanism of action, that is the modulation of monoaminergic neurotransmission at a synaptic level and, as a result, the range of depressed patients that the newer drugs treat is not better than the older antidepressant agents. Only approximately half of the patients treated with antidepressants show full remission of the disease, although many other show partial improvements in their symptoms [26]. The term treatment-resistant depression is typically used to describe a form of major depressive disorder that has not responded adequately to antidepressant treatment. Remission is the full resolution of depressive symptoms and should be the ultimate goal of treatment for depression. Remission is associated with lower levels of disability and dysfunction and a lower likelihood of relapse and recurrence. In the non-remitted patients, several treatment strategies are adopted including switching antidepressant treatment, combining antidepressants (from the same or from different pharmacological classes), or augmentation strategies that consist in the addition of a non-antidepressant treatment [59].

OBESITY AND METABOLIC SYNDROME

Overview

Obesity is defined as the excess body fat has accumulated to such an extent that health may be adversely affected [60]. Obesity is an important contributor to many of the chronic diseases that account for much of the morbidity observed worldwide [61]. The relationship between obesity and metabolic complications such as hypertension, coronary heart disease, ischemic stroke, dyslipidemia, type 2 diabetes and some cancers, is well defined [62,63].

Importantly, the amount of excess fat, its distribution within the body, and the associated health consequences vary considerably between obese individuals [60]. The increase of fat in the body is accompanied by alterations in physiological function, and these changes are dependent of the regional body fat distribution [64]. Interestingly, aging is associated with marked changes in body composition characterized by a reduction in free fat mass and an increase in fat mass that is greater in the intraabdominal compartment [65,66]. The excessive deposition of fat in the intra-abdominal compartment is associated with increased risk of the adverse health consequences of obesity [60]. Strong evidence support that abdominal fat is predictive factor of insulin resistance and metabolic abnormalities commonly referred to as the metabolic syndrome (MetS) [67].

Metabolic syndrome is a cluster of metabolic abnormalities associated with high risk of developing type 2 diabetes and/or cardiovascular disease. According to the International Diabetes Federation it is defined by the presence of visceral adiposity and at least two of the following criteria: hyperglycemia, dyslipidemia (high triglycerides and/or low high-density lipoprotein (HDL) cholesterol) and hypertension [68]. Visceral obesity is a central component of MetS and the aging process is associated with an increased deposition of body fat in the abdominal region [69].

MetS and MetS components are more prevalent in older than younger adults [65,70]. Age has been found to be associated with the way MetS is expressed and also with how different combinations of MetS components are associated with mortality risk [71]. While there is strong evidence for increased risk to individuals who arbor such metabolic abnormalities, it is unclear whether or not the association of these anomalies represents a single underlying pathogenic pathway [72].

Epidemiology

The World Health Organization (WHO) was described obesity as one of the most blatantly visible public-health problems, yet most neglected, that threatens to overwhelm both more and less developed countries [60].

Prevalence of overweight and obesity is increasing in every region of the world [73]. From 1980 to 2008 the prevalence of obesity has nearly doubled from 4.8% for men and 7.9% for women to 9.8% and 13.8% respectively [74]. Similarly, a study of overweight and obesity in children and adults from 1980-2013 found that worldwide, the proportion of overweight or obese increased in both developed and developing countries [75]. Projections to the year 2030 indicate that, after adjusting to secular trends, a total of 2.16 billion individuals will be overweight and 1.12 billion will be obese [76].

In Portugal data from the National Food, Nutrition and Physical Activity Survey (IAN-AF 2015–2016 Survey) indicate that the national prevalence of obesity is 22.3% and the prevalence of pre-obesity at national level is 34.8%. Importantly, the prevalence of abdominal obesity in adults is 50.5% and particularly high in the elderly reaching 80.2% [77].

The incidence of metabolic syndrome often parallels the incidence of obesity and incidence of type 2 diabetes. Global data on the prevalence of MetS is not available mainly because it is hard to measure; however, since MetS is about three times more common than diabetes, the global prevalence can be estimated to be about one quarter of the world population [78]. Data on the global prevalence of the features of MetS indicate that the prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [79]. The WHO estimates that in 2008 approximately 40% of adults aged 25 and above had been diagnosed with hypertension and the number of people with hypertension increased from 600 million in 1980 to 1 billion in 2008 [80]. Global data on raised triglycerides and low HDL levels is not available to our knowledge.

Estimates for the Portuguese population indicates that the prevalence of metabolic syndrome in adults was 49.6% using International Diabetes Federation definition. As expected, the most prevalent feature of metabolic syndrome was high blood pressure with 64.3% and the lowest was high fasting glucose that accounted for 24.9%. Consistent with data from the IAN-AF, abdominal obesity was present in 51% of sample, high triglycerides in 29.4% and low HDL cholesterol in 56.5% [81].

Clinical presentation

As mentioned, obesity is defined as the excess body fat has accumulated to such an extent that health may be adversely affected [60]. In clinical practice Body Mass Index (BMI – weight (kg)/height^2(m)) is generally used to classify obesity [64]. The WHO proposes that a BMI of 30 kg/m^2 or above is criteria to diagnose obesity and a BMI between 25 kg/m^2 and 29.9 kg/m^2. Table 1 presents the WHO classification of underweight, overweight and obesity in adults [60].

Table 1 - Classification of adults according to BMI.

Classification	BMI (kg/m^2)	Risk of comorbidities
Underweight	<18.50	Low (but risk of other clinical problems increased)
Normal range	18.50-24.99	Average
Overweight	≥ 25.00	
Preobese	25.00-29.99	Increased
Obese class I	30.00-34.99	Moderate
Obese class II	35.00-39.99	Severe
Obese class III	≥40.00	Very severe

BMI has been widely used in clinal and research settings. For adults, BMI values are age-independent and equal for genders. The usefulness of these index is well established: it allows for meaningful comparisons of weight status within and between populations, for the identification of individuals and groups at increased risk of morbidity and mortality, to identify and establish priorities for intervention at individual and community levels and constitutes a firm basis for evaluating interventions [60]. Although useful, this adiposity index is not exempt from limitations. BMI calculation does not distinguish lean muscle from fat mass; therefore, BMI can result in inaccurate assessment of adiposity. BMI also does not take into consideration sex differences in the distribution of fat [82]. Aging is associated with considerable changes in body composition characterized by a progressive decrease in fat-free mass and increase in fat mass. Also, aging is associated with a redistribution of both body fat and fat-free mass characterized by an accumulation of intraabdominal fat [66]. BMI does not take into consideration the body fat distribution and it was demonstrated that central obesity (with excessive visceral fat) was closely related with the

common complications of obesity, such as insulin resistance, atherogenic dyslipidemia, type 2 diabetes, and cardiovascular disease [83].

Considering the above-mentioned limitations, other methods in addition to BMI would be valuable in identifying individuals at increased risk from obesity-related morbidity due to abdominal fat accumulation. Waist circumference (WC) is a convenient and simple measurement that is unrelated to height, correlates closely with BMI and is an approximate index of intra-abdominal fat mass and total body fat. Furthermore, changes in waist circumference reflect changes in risk factors for cardiovascular disease and other forms of chronic disease [60]. Table 2 presents the waist circumference and risk of metabolic complications associated in in adult Caucasians accordingly to WHO [60].

Table 2 – Sex-specific waist circumference and risk of metabolic complications associated with obesity in Caucasians.

Risk of metabolic complications	Waist circumference (cm)	
	Men	Women
Increased	≥94	≥80
Substantially increased	≥102	≥88

Strong evidence support that abdominal fat is predictive factor of insulin resistance and metabolic abnormalities commonly referred to as the MetS [67]. Evidence that central obesity, as assessed by waist circumference, was an essential component of MetS due to the evidence linking waist circumference with cardiovascular disease and the other metabolic syndrome components, and the likelihood that central obesity is an early step in the etiological cascade leading to full MetS [68]. Other established components of MetS are increased serum triglyceride levels, decreased serum HDL cholesterol levels, high blood pressure, and impaired fasting glucose [68,84]. The International Diabetes Federation diagnostic criteria for MetS is presented in Table 3.

The ultimate importance of diagnosing MetS is that it helps identify individuals at high risk of both type 2 diabetes and cardiovascular disease [68]. Several concerns have been raised regarding the utility of MetS diagnosis because it may be considered useful as an educational concept; although, it has limited practical utility as a diagnostic or management tool [84,85]. For instance, the cardiovascular disease risk

associated with MetS appears to be no greater than the sum of its parts and the treatment of MetS is no different than the treatment for each of its component [84]. Furthermore, MetS definition has several inherent limitations. Diagnosis of MetS is a dichotomous classification based on the binary identification of the risk factors used to define MetS. Diagnostic criteria for MetS do not include all the risk factors and cardiovascular risk varies according to the risk factor combination used to diagnose MetS in an individual. Also, individuals diagnosed with MetS constitute a heterogeneous metabolic group since diagnosis can be made using completely different criteria [84,85].

Table 3 – Metabolic syndrome definition accordingly International Diabetes Federation [68].

MetS components	Criteria for diagnosis
Central obesity [a)]	Waist circumference Men ≥94 cm; Women ≥80 cm
Plus any two:	
Raised triglycerides	>150 mg/dL or specific treatment for this lipid abnormality.
Reduced HDL-cholesterol	<40 mg/dL in men; <50 mg/dL in women or specific treatment for this lipid abnormality.
Raised blood pressure	Systolic ≥130 mm Hg or Diastolic ≥85 mm Hg or treatment of previously diagnosed hypertension.
Raised fasting plasma glucose [b)]	Fasting plasma glucose ≥100 mg/dL or previously diagnosed type 2 diabetes.

[a)] If BMI is over 30 kg/m^2, central obesity can be assumed. Cut-offs presented for Europids, other ethnic-specific cut-offs are available.

[b)] If above 100 mg/dL, oral glucose tolerance test is strongly recommended, but is not necessary to define presence of syndrome. In clinical practice, impaired glucose tolerance is also acceptable.

Etiology and mechanisms

Obesity is a heterogeneous group of conditions with multiple causes rather than a single disorder [64]. Excessive body weight and adiposity are the result of physiological, genetic, environmental, behavioral, social, and cultural factors that result in energy imbalance and promote excessive fat deposition [64,86]. The relative contribution of each of these factors has been studied extensively. Genetic factors may play an important role in the regulation of body weight; although, the WHO Consultation on Obesity concluded

that behavioral and environmental factors (sedentary lifestyles combined with excess energy intake) are primarily responsible for the dramatic increase in obesity during the past 2 decades [60,86].

It was estimated that genetic factors account for 50% to 90% of the variability in BMI [87]. While genetic factors may be of relevance to the etiology of obesity, their impact may be exacerbated or attenuated by environmental factors. The genetic influences in obesity seem to work through susceptibility genes which may increase the risk of developing obesity but are not essential for its expression or are not enough by themselves to explain obesity. Several candidate genes have been associated with human obesity or its metabolic complications and include receptors that are important in mechanisms of thermogenesis as well as those involved in appetite regulation [64,86]. It is now accepted that the contribution of genetic factors to obesity are due to the combined effect of many risk variants in different genes [88].

Despite the influence of genetics in the regulation of body weight, the rapidity escalation of obesity prevalence worldwide has suggested that genetic factors cannot play the predominant role in the current obesity epidemic. Therefore, behavioral and environmental factors are largely responsible for the increase in the prevalence of obesity. Currently, obesity epidemic appears to be the result of environmental and behavioral factors interacting with genetic susceptibility [86].

Overweight and obesity are the result of positive energy balance. This may be seen as an excess of energy intake relative to daily energy expenditure, or as low energy expenditure relative to daily energy intake. Low levels of physical activity and dietary behaviors that exceed the recommendations are thought to contribute to the etiology of obesity. High energy intakes have been associated with high fat intake and energy-dense foods, intake of sugar-sweetened drinks, low intake of foods and nutrients that may have appetite-controlling properties (i.e. fruit and vegetables, fiber) and meal patterns that interfere with the regulation of energy intakes [89]. Physical activity has an impact on energy expenditure and is a major determinant of energy balance. Technological advances and industrialization, such as vehicles, elevators, escalators, and other labor-saving machines, enabled humans to become highly sedentary individuals [86].

The exact etiology underlying MetS is still not completely understood. Many contributing factors and mechanisms have been proposed, including obesity, adipose tissue dysfunction, insulin resistance, chronic inflammation and genetic factors [84,90]. Obesity, particularly visceral obesity, seems to be one of the main driving factors of MetS by increased production of free fatty acids whose activity, in turn, might interfere with the action of insulin [91]. Obesity has been considered as an endocrine and inflammatory

disorder intimately related with insulin resistance because adipose tissue is able to secrete many humoral substances, such as TNF-α, leptin, adiponectin, resistin, and visfatin among others [84].

Insulin resistance seems to be one of the major underlying mechanism responsible for the MetS. Insulin resistance in the central nervous system is the main cause of obesity by regulating appetite and food intake behavior. Furthermore, insulin resistance in adipose tissue results in hyperlipidemia and inflammation; hepatic insulin resistance causes hyperglycemia; cardiac insulin resistance promotes heart failure; pancreatic insulin resistance results in impaired β-cell regeneration; insulin resistance in vascular endothelium promotes hypertension and disrupts glucose homeostasis [92].

Chronic disturbance of metabolic homeostasis, like the one that occurs in overnutrition, could lead to aberrant immune responses. Obesity, insulin resistance and type 2 diabetes are closely associated with a chronic inflammatory response characterized by abnormal cytokine production, increased acute-phase proteins and activation of a network of inflammatory signaling pathways. Oxidative stress has been linked to diabetic complications through endothelial dysfunction. Recent evidence indicates that oxidative stress and mitochondrial dysfunction also have important roles in type 2 diabetes. These pathways are also coupled to the activation of inflammatory pathways and insulin resistance in adipocytes and muscle cells, and impaired insulin secretion in pancreatic β-cells [93,94].

Clustering of the MetS in relatives suggests a genetic component. Each one of the MetS components has a genetic basis for which candidate genes have been identified [95]. Some genes, which have ensured optimal storage of energy during periods of fasting, may contribute to the etiology of MetS. Common variants in candidate genes influencing fat and glucose metabolism can, together with environmental factors, increase susceptibility to MetS. Among the genes that may increase the risk of the MetS we can find the ones that encode for β_3-adrenergic receptor, hormone-sensitive lipase, lipoprotein lipase, IRS-1, skeletal muscle glycogen synthase among others [96].

A significant body of scientific literature indicates that the dysregulation of the HPA axis may play a role obesity and MetS. Alterations of the HPA axis in abdominal obesity are associated with insulin resistance, which suggests a direct responsibility of these hormonal alterations in the susceptibility to develop both metabolic and cardiovascular diseases which are features of MetS [97,98]. Animal models exposed to chronic physical and psychological stress present high visceral fat deposition, insulin resistance, hyperinsulinemia, impaired glucose tolerance, adrenal hypertrophy, enhanced cortisol response to ACTH stimulation, altered lipid profiles, and greater incidence of coronary artery atherosclerosis [98].

Bariatric surgery

Several treatment options are available to treat obesity and may include behavioral, pharmacotherapeutic, and surgical approaches or a combination of any of those [99]. As previously mentioned, treatment of MetS is no different than the treatment for each of its component [84]. For the purpose of this thesis we will only address bariatric/metabolic surgery, specifically Roux-en-Y gastric by-pass (RYGB) and sleeve gastrectomy (SG).

Currently, bariatric surgery is the best method of achieving substantial sustained weight loss in severely obese [100,101]. The most recent data from the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) indicates that SG and RYGB are the most frequently performed types of bariatric interventions accounting for 85.5% of all the bariatric surgery procedures. SG increased from 37% in 2013 to 45.9% in 2014, overcoming RYGB as the most performed bariatric surgery worldwide. On the other hand, RYGB decreased from 45% in 2013 to 39.6% in 2014 [102].

In the last years the numbers of bariatric surgery procedures have increased due to numerous factors. The growing rate of obesity that has led to more individuals to seek treatment is one of the reasons. The obesity-related mortality and comorbidities are also responsible for patients seeking treatment through bariatric surgery. The long-term efficiency of bariatric surgery compared to nonsurgical treatments can be also pointed as one reason. Technologic advances in bariatric surgery procedures, such as introduction of laparoscopic approaches, have improved safety and facilitated shorter hospital stays. The increasing awareness of the procedure among patients and health care professionals, have influenced the continued rise in bariatric surgical procedures conducted over the years [103].

Based on the supposed mechanism of weight loss, bariatric surgery procedures were initially classified as restrictive, malabsorptive, or combined. RYGB is a combined procedure that incorporates both restrictive and malabsorptive elements and is the current gold standard treatment for severe obesity [104]. The RYGB includes a small gastric pouch with 15 to 30 mL on the lesser gastric curvature which is completely separated from the gastric remnant and then anastomosed to the jejunum, leaving an alimentary or Roux limb of typically 100 to 150 cm. Bowel continuity is restored by an entero-entero anastomosis between the excluded biliopancreatic limb and the alimentary limb [105]. SG is a restrictive bariatric surgery technique that consists of subtotal vertical gastrectomy with preservation of the pylorus, including longitudinal resection of fundus, corpus and antrum, that creates a tubular duct along the lesser

curvature. Resection comprises approximately 80% of the stomach and the remnant gastric has a capacity of approximately 100 mL. It is considered an easier technique than other procedures such as RYGB. Its good results have led to a rise in its use, and it is currently one of the most performed technique worldwide [106].

Most of the comorbidities of obesity show a reversal or amelioration after bariatric surgery. Several studies offer evidence that improvement of metabolic abnormalities, such as type 2 diabetes mellitus, can be achieved as a consequence of bariatric surgery [107]. Bariatric surgery was also shown to decrease the low grade inflammation associated with obesity as measured by CRP and IL-6 levels [108]. Many of the metabolic improvements observed after bariatric surgery occur with little or no weight loss. Therefore, concepts of restriction and malabsorption do not fully explain the effects of bariatric surgery [101].

Alterations in the hormonal milieu have been receiving attention to explain the mechanisms of action of bariatric surgery. Leptin and insulin are important hormones that act in the central nervous system to influence long-term energy balance and decrease following all forms of bariatric surgery. Adiponectin rises following bariatric surgery and in response to energy restriction and weight loss. RYGB leads to a rapid rise in meal stimulated glucagon-like peptide 1 (GLP-1) and peptide YY blood levels. GLP-1 provides a rapid incretin response and fullness after small meals, actions that are thought to be crucial in the early improvement in type-2 diabetes and the weight loss efficacy of this procedure. There is also evidence that SG stimulate some increase in GLP-1 and peptide YY in response to a meal. Following RYGB, changes in both fasting and meal response ghrelin levels are conflicting, with reports of substantial lowering, no change, and higher concentrations. Ghrelin levels are clearly reduced following SG, through removal of the gastric fundus. Several other hormonal alterations have been studied in the context of bariatric, yet results are conflicting or no sufficient data has been generated to establish a pattern of change [101].

Obesity and mood

Depression and obesity have increased in parallel in the last decades [25]. Both conditions are associated with an increased risk of mortality and morbidity, reduced quality of life and disability [109]. Several systematic reviews and meta-analytic studies have shown that depressive mood and depression are associated with obesity [1-5].

Interestingly, mounting evidence suggests a bidirectional association between obesity and depression [110,111]. In agreement, several studies suggest that these conditions are linked through a vicious cycle of adverse physiological adaptations that are mutually reinforced [111].

HPA axis is dysregulated both in obesity and depression. In depression, neurons in the paraventricular nucleus of the hypothalamus secret CRH that stimulates the synthesis and release of ACTH from the anterior pituitary that will in turn stimulate the synthesis and release of glucocorticoids from the adrenal cortex. Under normal conditions, a negative feedback mechanism of the glucocorticoid receptors is activated and downregulates the cortisol response; however, an excessive activity of the HPA axis will lead to a decrease negative feedback and sustained elevated levels of glucocorticoids [29]. Glucocorticoid receptors are highly expressed in visceral adipose tissue (VAT) and cortisol facilitates the accumulation of lipids on those tissues by activation of the glucocorticoid receptors [112]. This is one of the possible links between depression and obesity.

Another possible link between depression and obesity is inflammation. Meta-analyses of cross-sectional and longitudinal studies have shown an increased inflammatory response in patients with MDD [52,113]. HPA activation suppresses pro-inflammatory response in threatening conditions, when exposure to actual or perceived danger is maintained, the HPA axis promotes an increase in inflammatory response [112]. Circulating proinflammatory cytokines are able to directly and indirectly stimulate the HPA axis [114] and the increased production of pro-inflammatory cytokines has also been associated with glucocorticoid resistance and hyperactivity of the HPA-axis [113]. Therefore, inflammation will promote abdominal fat deposition through indirect effects on the HPA axis. In turn, obesity can cause a state of low-grade inflammation. Adipose tissue produces and releases a variety of pro-inflammatory and anti-inflammatory factors, including adipokines, such as leptin, adiponectin, and resistin, and cytokines, such as TNF-α, IL-

6. Overproduction of pro-inflammatory mediators results in inflammation of the adipose tissue that spreads to overall systemic inflammation [115].

Other factors may also link obesity and depression. For instance, unhealthy lifestyles are also related to mental health. People with depression have a higher prevalence of excessive alcohol drinking and are physically more inactive [116], which may be associated with obesity. Stress may be a trigger for depression that and may result in undereating or overeating. The way stress affect eating seems to be influenced by stressor severity and duration. Chronic stress seems to be associated with a greater preference for energy-dense foods, particularly foods that are high in sugar and fat. Evidence from longitudinal studies suggests that chronic life stress may be causally linked to weight gain and stress-induced eating may be one factor contributing to the development of obesity [117,118].

There are many physical, economic, social, familial, emotional and behavioral consequences of obesity. One common psychological impact of obesity is the impairment in the perception of body image. People with negative body image are more likely to have an eating disorder and suffer from negative emotions such as depression, sadness, isolation and loss of self-confidence [119]. Furthermore, obesity is associated with several health complications and it is known that depressive symptomatology may occur in the context of medical illness [120]. These factors may be part of a vicious circle that potentially promote and potentiate both depression and obesity.

In younger to middle-aged individuals, when underweight conditions are excluded, depression and obesity are positively associated in a "dose-dependent" manner [1,2,109,121]. In a study by de Wit *et al.* [109] depression was found to be associated with the quadratic term of BMI (BMI^2) indicating a U-shaped association. Individuals classified as underweight and obese, according to BMI, presented a higher depressive symptomatology than the ones with normal weight and pre-obesity. McCrea et al. [122] also found a U-shaped association between mental disorders and BMI in men. The association of depression and obesity was examined in a meta-analysis of cross-sectional studies that included 204,507 participants and in a meta-analysis of longitudinal studies that included 58,745 participants [1,2]. In both cases a positive link between depression and obesity was observed; furthermore, in the meta-analysis of longitudinal studies obesity was found to increase the risk of depression and depression was found to be predictive of developing obesity.

While in general population the positive association between obesity and depression seems to be well established, in older ages, observations from several studies revealed contradictory results. While some

studies, based in BMI as a measure of adiposity, support the hypotheses that higher adiposity is associated with less depressive mood [123-127], other studies that complement BMI with WC and VAT, demonstrate a positive association of adiposity and depressive mood [128-131]. A positive association of VAT, but not subcutaneous adipose tissue (SAT), with depressive mood was observed in middle age individuals [56,130,132]. Methodological issues are likely to justify some of the discrepancies reported, particularly since the anthropometric methods to assess obesity in the clinical setting (BMI and WC) are not able to discriminate the pattern of adipose tissue distribution and given the age-associated body fat redistribution, the lack of accuracy can possibly be more pronounced in the elderly [66,133]. It was previously highlighted that age and gender could be key components in the association of obesity and depression. In the English 2007 Adult Psychiatric Morbidity Survey, a study that included participants with 16 and more years of age it was found that the association of obesity and depression diminished in older age groups, particularly when potential confounders such as physical health were taken into account [122]. Understanding the role of age and body fat distribution seems to be paramount for the design of better prevention strategies and design of interventions.

Metabolic syndrome and mood

Similar to obesity, MetS has also been associated with depression in a positive and bidirectional manner. Vancampfort *et al.* [12], in a meta-analysis that included 5,531 subjects, found that individuals with MDD had a higher MetS prevalence. Pan and collaborators [10], in a meta-analysis that included 155,333, subjects demonstrated that MetS was associated with depression; furthermore, baseline MetS could predict the risk of developing depression, and that the reverse is also true.

MetS is not a clinical entity, rather a constellation of metabolic abnormalities that increase the risk of developing type 2 diabetes and/or cardiovascular disease. Potential mechanisms involved in the association between depression and MetS are complex and may involve several shared physiological pathways. To understand the pathophysiology that mediates the association between MetS and depression is therefore necessary to understand the association of its components and depression. Central obesity is a risk factor and a central component of MetS and the possible mechanisms linking obesity and depression were previously explored. In addition to obesity, insulin resistance is other major underlying mechanism for the MetS that has also been mechanistically and epidemiologically associated with depression [92,134]. Insulin receptors are expressed throughout the brain and are expressed in regions

classically involved with mood regulation, such as the amygdala, nucleus accumbens and ventral tegmental area. Insulin has a central role and has been implicated in modulating feeding behavior and energy maintenance by the hypothalamus and in memory-related processes by the hippocampus [134]. Evidence from animal models with brain-specific knockout of insulin receptor (NIRKO) was shown to cause age-related anxiety and depressive-like behavior, altered mitochondrial function, aberrant monoamine oxidase expression, and increased dopamine turnover in the mesolimbic system [50]. Insulin promotes neuroplasticity in the developing and adult brain and insulin resistance possible contributes to neuroplasticity deficits in obesity and type 2 diabetes mellitus [135]. Insulin secretion is reduced in conditions of high cortisol concentrations, at the same time, insulin elevates adrenocorticotropin and corticosterone hormone levels, promoting HPA axis activation [134,136]. Insulin has an anti-inflammatory role and resistance to its anti-inflammatory actions could result in enhanced circulating levels of proinflammatory cytokines resulting in persistent low-grade inflammation. In addition, inflammation and cytokines that are highly expressed in depression, such as TNF-α, may induce insulin resistance through serine phosphorylation of insulin receptor substrate 1, decreasing the tyrosine kinase activity of the insulin receptor and the intracellular insulin signaling [137].

Another potential explanation is that vascular damage in the brain might predispose to depression in the elderly according to the vascular depression hypothesis. MetS, as a cluster of vascular risk factors, could lead to subclinical vascular damage, which in turn may produce depressive symptoms. In turn, a meta-analysis found that depression increased the risk of hypertension incidence [138].

Behavioral and psychological factors may also link MetS and depression. MetS is associated with a sedentary lifestyle and a negative self-perception due to stigmatization of obesity, a component of MetS, which can lead to an increased risk of depression [139]. Furthermore, depressed individuals tend to present a poor diet, sleep disturbances and engage in less physical activity, all of which are known risk factors for the development of MetS [10].

Several cross-sectional studies have associated depression or depressive symptoms with various components of MetS. As previously explored, in meta-analyses of cross-sectional and longitudinal studies, obesity, a central component of MetS, was found to be positively associated with depression [1,2]. Vancampfort *et al.* [12], also found that MDD patients had a higher risk for hyperglycemia. Further supporting those results Kan *et al.* [6] found a small but significant cross-sectional association between depression and insulin resistance. Anderson *et al.* [7] described that the presence of diabetes doubles the

odds of comorbid depression. In another meta-analytic review, which included subjects with type 1 or type 2 diabetes, depression was associated with poor glycemic control [8]. Furthermore, positive association between depressive symptoms and insulin resistance was found in a large community based study [140].

Findings regarding dyslipidemia have been inconsistent with positive, negative or null associations between total cholesterol and depressive mood having been reported. An association between lipid components and depressive mood has been hypothesized, but published results are inconsistent [141]. Low HDL cholesterol, and a higher atherogenic index (total/HDL cholesterol and LDL/HDL cholesterol) was associated with major depression [142]. In one longitudinal analysis, a higher total to HDL cholesterol ratio was associated with a faster increase in depressive symptoms among women [141]. In other studies, higher triglyceride levels were observed in patients with ongoing major depression than in those in remission or controls [143,144]. Vancampfort *et al.* [12], also found that MDD patients had a higher risk for hypertriglyceridemia.

Contrary to what would be expected, in a large cross-sectional study and in a prospective study of aged individuals low blood pressure was associated with depressive mood [145,146]. Hildrum *et al.* [147] found an association of low blood pressure with anxiety and depression. In a longitudinal study, the same authors, also found that symptoms of anxiety and depression predicted lower blood pressure 11 years later [148]. Despite the amount of research suggesting a negative association between depression and blood pressure, results have been conflicting. In a twin's study, Scherrer *et al.* [149] reported that hypertension was significantly associated symptoms of depression. In a review of the literature, Rutledge *et al.* [150] found that psychological factors, such as anger, anxiety, and depression were predictors of hypertension development. Conciliating these results, Licht *et al.* [151] showed that depressive disorder was associated with low systolic blood pressure and less hypertension, whereas the use of certain antidepressants was associated with both high diastolic and systolic blood pressures and hypertension.

Despite the significant amount of literature exploring the association of depression or depressive symptomatology with MetS or its components several questions remain to be answered. A significant obstacle to the evaluation of the association between MetS and depressive mood is the fact that individuals diagnosed with MetS constitute a heterogeneous metabolic group, grouping in the same condition individuals that may present different metabolic profiles. Furthermore, the contribution of each of the components of the overall syndrome differs between individuals and the weight of the MetS components to the association with mood has yet to be determined. Also, the severity of the metabolic

abnormality observed in each component may be relevant for the degree of association and the classification of MetS does not allow the exploration of that contribution. MetS tends to be more prevalent with the advance of age and at the same time depressive symptomatology has a greater impact in older ages; therefore, the role of age in the association needs to be understood. Finally, the association of MetS and depression should be reflected at a neural level and scientific literature exploring the neural correlates of that association is scarce or absent.

Bariatric surgery and mood

Bariatric surgery provides an excellent model to understand the effects of obesity and comorbidity on neurocognitive function and brain health, given the weight loss and resolution of comorbid conditions represent a novel change [152].

As previously mentioned, obesity is associated with depression and depressive mood [1,2]. Considering that positive association, is not surprising that several lines of research indicate that the prevalence of mood disorders, mostly depression are more prevalent in preoperative bariatric surgery patients [153]. Anxiety in bariatric surgery candidates is less studied and understood than depression and depressive mood. Clinically significant anxiety is common among bariatric surgery candidates and anxiety symptoms are more common than what has been hypothesized [154].

Interestingly, it has been demonstrated that weight loss surgery is associated with an improvement in psychological status [153,155-157]. These findings are particularly interesting in the case of anxiety and depression. For those conditions, improvement might be termed dose-dependent, meaning that larger excessive weight loss is associated with greater improvement in symptoms [155]. Surprisingly, despite the reported improvements in anxiety, depression and general mental health of bariatric surgery patients, this type of procedures has also been associated with an increased risk of suicide and self-harm emergencies [158,159]. A systematic review suggested that suicide risk for patients undergoing bariatric surgery is 4 times higher than the general population norm [160]. A possible explanation on the deleterious effects of bariatric surgery observed in those patients may be related to changes in body image, diet-related stress, and unmet expectations of weight loss that might increase mental health problems. Despite the body of literature on the topic, there is substantial uncertainty about whether bariatric surgery has positive or negative effects on mental health [158].

Adverse neurocognitive outcomes of obesity are not restricted to anxiety and depression. Obesity has also been linked to cognitive impairments and increased risk of dementia [152]. A systematic analysis of scientific literature revealed that obese adults may present impairments across almost all cognitive domains, such as complex attention, verbal and visual memory and decision making [161]. Furthermore, morbidly obese patients seeking bariatric surgery may also present cognitive deficits [162]. The relevance of cognitive function is further reinforced by the fact that deficits in memory and executive function may contribute to poorer adherence to post surgery guidelines and impair weight loss and the results of operation [163]. There is increasing evidence that weight loss following bariatric surgery is associated with sustained improvements in memory, executive function and cognitive control [164].

The mechanisms of action by which bariatric surgery works are still an enigma. Moreover, the mechanisms underlying mental health improvement and cognitive gains after bariatric surgery have yet to be identified [152]. Obesity comorbidity, such as insulin resistance, type 2 diabetes, hypertension and sleep apnea, have been associated with depression and cognitive function [6-9,165-167]. These obesity-associated medical conditions tend to improve or remit after bariatric surgery [168-170] which could be a potential explanation for the neurocognitive improvements observed. Elevated inflammation is a common feature of obesity and is associated with cognitive impairments and depression, in turn bariatric surgery is associated with a decrease in inflammatory makers, implicating it as a possible mechanism for neurocognitive improvement [152,171].

More recently, hormonal changes have been implicated in the explanation of the results of bariatric surgery [101,172]. The most well studied hormones in the relevant studies are leptin, ghrelin and insulin. While several of these hormones, such as leptin and insulin, have been shown to fall after all forms of bariatric surgery [101,173,174], others, such as ghrelin, have been less predictable [101,174,175]. Beyond their effects on appetite regulation and metabolism, these hormones have been studied as potential mediators of the observed association between adiposity and psychological [176] or cognitive function [177,178]. Alosco *et al.* [179] have shown that improvements in leptin and ghrelin levels following bariatric surgery appear to contribute to postoperative cognitive benefits. Several other hormones may play an important role in the neurocognitive outputs of bariatric surgery. Significant metabolic/hormonal alterations are induced by bariatric surgery that might affect anxiety, mood and cognition. After bariatric surgery, metabolic biomarkers, anxiety, mood and cognition all are recognized to vary with time. The variation observed over

time may indicate that the associations between these factors are dynamic; however, that hypothesis has not been tested.

Antidepressants and appetite

Antidepressants may have an important impact in the relation of mood with obesity and metabolic dysfunction. In humans and in rodents, antidepressant treatment has been shown to reduce HPA activity [180]. Also, antidepressants, particularly SSRIs and serotonin and norepinephrine reuptake inhibitors, have anti-inflammatory effects that may have been underestimated as target for the treatment of depression [181]. As mentioned earlier, both HPA hyperactivity and inflammation, have been implicated in mood disorders, obesity and metabolic dysfunction; therefore, it would be expected that obesity and metabolic dysfunction can be modulated by antidepressant treatment.

Surprisingly, evidence from meta-analytic data indicates that amitriptyline mirtazapine and paroxetine are associated with weight gain [182]. Several other antidepressants have also been associated with weight gain; yet, the underlying mechanisms that link antidepressant use and weight gain are not fully understood [25]. Evidence also indicates that antidepressant treatment may induce weight gain by acting in central mechanisms regulating appetite and food intake [182]. SSRIs may be relevant on this aspect since serotonin plays an important role in eating behavior and preference for certain macronutrients, such as carbohydrates [183]. Furthermore, previous studies show that weight gain associated with antidepressants may reflect their action on monoamine pathways, which include serotonergic and noradrenergic receptors, among others [25].

Contrary to other psychiatric disorders such as schizophrenia [184,185] and eating disorders [186-188] or medical conditions like obesity [189,190] or Prader-Willi syndrome [191], a survey of the literature reveals the amount of research exploring the neural correlates of appetite changes in the context of depressive episodes is surprisingly scarce and recent [192]. Few studies have explored the association of appetite brain activity in the context of depression [192-194]. Only one study [194] explored the patterns of brain activity in response to food stimuli in depressed patients and in controls with increased or decreased appetite. The other works [192,193] provide the first clues regarding the association of appetite neural correlates with endocrine, metabolic and inflammatory alterations in depressed patients. Also, while for schizophrenia [185,195] and obesity [196] there are studies exploring the impact of pharmacological treatment in appetite related

neurocircuitry, to the best of our knowledge, no study has been published exploring the effects of antidepressants in appetite neurocircuitry of depressed patients.

Paroxetine is a well-tolerated, high affinity and potent SSRI that is effective in the treatment of both depressive and anxiety disorders across the age range [197]. Despite being well-tolerated, paroxetine treatment induces more weight gain than other SSRIs [198]. Interestingly, paroxetine has been associated with a marginal weight loss during acute treatment but associated with significant weight gain when used over longer periods [182]. Due to its effects on weight, paroxetine may be an interesting drug to initiate the exploration of the effects of SSRIs in appetite, body composition and metabolism. This is a new field that deserves exploration given the understanding of the effects of antidepressants on appetite will potentially illuminate the path toward new interventions targeting the development of depression-related obesity, and its concomitant illnesses in patients under antidepressants.

CONTEXT OF THE WORK AND AIMS

It is now clear that a relationship of mood with obesity and metabolic syndrome exists. Those associations are further reinforced by possible biological mechanisms and pathways that are not yet clear or fully understood. Understanding these relationships can open new routes to target modifiable factors that can influence both mood and metabolic disturbance.

In younger to middle-aged individuals, depression and obesity are positively associated; however conflicting results have been published regarding the association between mood and obesity in old age. Depressive symptoms are widespread in the elderly, with subthreshold depression being more prevalent than major depression and, considering the alterations in body composition that occur with aging it's important to understand the effects of age in this association. Since metabolic dysfunction is closely related with obesity and metabolic syndrome is more prevalent at older ages understanding the association between depressive mood and metabolic dysfunction and its brain correlates is also important. The treatment of obesity with bariatric surgery offer an important opportunity to explore the temporal dynamics between mood, cognition and metabolic biomarkers that are still unexplored. Furthermore, the impact of antidepressant treatment brain correlates of appetite and alter body composition is also unknown.

Taking this into consideration we aimed to explore new perspectives on the interplay between mood and metabolism in the context of aging, obesity and depression.

More specifically we aim to address:

- Explore the influence of age in the association between adiposity and depressive mood.
- Understand the relationship between metabolic dysfunction and depressive mood and its impacts at central level.
- Investigate the temporal dynamics of the association between metabolic biomarkers with depressive mood and cognition after bariatric surgery.
- Study the impact of depression and its pharmacological treatment in appetite and body composition.

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CHAPTER II

The moderator effect of age in the association between mood and adiposity in the elderly is specific for the subcutaneous adipose compartment: an MRI study.

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(Article published in the International Journal of Geriatric Psychiatry, 2019, Oct 27.

doi: 10.1002/gps.5226 [Epub ahead of print])

The moderator effect of age in the association between mood and adiposity in the elderly is specific for the subcutaneous adipose compartment: an MRI study.

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ABSTRACT

Objectives: The positive association between obesity and depressive mood in young and middle age individuals is a phenomenon with major clinical implications in public health. Interestingly, the trend of this association in older individuals is not clear, given the conflicting results of multiple studies. Since aging is accompanied by changes in body fat distribution, we questioned whether age is a modulator of such association. This study explores the role of age in the association between mood and general (BMI) and abdominal adiposity (waist circumference - WC) in older adults characterizing the different abdominal adipose tissue compartments (subcutaneous adipose tissue - SAT and visceral adipose tissue - VAT) with magnetic resonance imaging techniques (MRI).

Methods: 120 aged community-dwelling individuals, (≥50 year of age), were assessed regarding depressive mood (Geriatric depression scale), and adiposity (BMI and WC). From these, 96 were assessed for SAT and VAT using MRI.

Results: Using multiple linear regression models depressive mood was positively associated with BMI, WC and VAT. Age was a significant moderator of the association between depressive mood and BMI, WC and SAT: positive in younger participants and null or negative the older. On the other hand, higher VAT was significantly associated with a more depressive mood, independently of age.

Conclusions: This study identifies age as a relevant moderator in the association between depressive mood and adiposity in the elderlies. Furthermore, the body fat compartment analysis revealed that the effect of age is specific for the SAT, suggesting its protective role in depressive mood.

Key-words: Visceral Fat; Subcutaneous Adipose Tissue; Mood Disorders; Age; Magnetic Resonance

INTRODUCTION

The rise in life expectancy in the last century has led to a significant increase of the elderly worldwide [1]. Aging is associated with higher prevalence of chronic diseases, namely metabolic syndrome and obesity [2-4]. Obesity is a well-known risk factor for several medical conditions, including the pathologies that compose the metabolic syndrome, but also for psychiatric conditions, namely depression [5].

Depression is one of the most prevalent psychiatric disorders in older individuals [6] and is associated decreased quality of life, functional decline, marked disability, and higher mortality from comorbid medical conditions [7,8] It is estimated to become the leading cause of disease burden in middle and higher income countries by the year 2030 [9].

Interestingly, mounting evidence suggests a bidirectional association between obesity and depression [10,11]. In agreement, several studies suggest that these conditions are linked through a vicious cycle of adverse physiological adaptations that are mutually reinforced. Therefore, it is essential to search for determinants that may modulate/interfere with the interplay between both conditions [11].

In younger to middle-aged individuals, when underweight conditions are excluded, depression and obesity are positively associated in a "dose-dependent" manner [5,12-14]. However, in older ages, observations from several studies revealed contradictory results. While some studies, based in body mass index (BMI) as a measure of adiposity, support the hypotheses that higher adiposity is associated with less depressive mood [15-19], other studies that complement BMI with waist circumference (WC) and visceral adipose tissue (VAT), demonstrate a positive association of adiposity and depressive mood [20-23]. A positive association of VAT, but not SAT, with depressive mood was observed in middle age individuals [22,24,25]. Of notice, gender and socioeconomic status seem to influence the obesity-mood association. Interestingly, effects of age seem to be inconsistent [12], particularly in the elderly [26,27] . Methodological issues are likely to justify some of the discrepancies reported, particularly since the anthropometric methods to assess obesity in the clinical setting (BMI and WC) are not able to discriminate the pattern of adipose tissue distribution [28]. Given the age-associated body fat redistribution, the lack of accuracy can possibly be more pronounced in the elderly.

Considering the alterations in body composition that occur with aging, we hypothesized that the association between adiposity and mood can change with age. The present study aimed to further investigate if, in older adults, age is a moderator in the association between depressive mood and adiposity and to test if this effect is present in the association with SAT and VAT.

SUBJECTS AND METHODS

Ethics statement

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

Study sample

Subjects

The participants included in the present study are part of the sample recruited for the SWITCHBOX Consortium project (www.switchbox-online.eu/). Briefly, the recruitment was performed in two-phases. In the first phase, participants (n = 1051, final sample size after exclusion criteria; males and females, 50+ years of age) were randomly selected from the Guimarães and Vizela local area health authority registries. For age and gender, the distribution of this database differs in less than 2% of that of the distribution for the Portuguese population estimated by the Portuguese authority on statistics, the "Instituto Nacional de Estatística" [29]. All participants were community-dwellers. The primary exclusion criteria included inability to understand informed consent, participant choice to withdraw from the study, incapacity and/or inability to attend the clinical and neuropsychological assessment session(s), dementia and/or diagnosed neuropsychiatric and/or neurodegenerative disorder (medical records). A team of experienced clinicians performed a standardized clinical interview that also addressed current medication and allowed to further detect and exclude disorders of the central nervous system (epilepsy and neurodegenerative disorders) as well as overt thyroid pathology [30,31].

In the second recruitment phase, 120 subjects, representative of the previous sample for gender, age, cognitive and mood profile, were randomly selected (Figure 1A) [32]. Socio-demographic characteristics (including years of formal education and occupational status) were collected.

Mood, lifestyle and body composition assessment

The Geriatric Depression Scale; long version (GDS) [33] was administered to provide a measure of depressive mood status.

Lifestyle variables, including as physical activity, smoking habits and alcohol consumption, were collected as previously reported [31]. Briefly, physical activity categories were: none, less than 3, over 3 times per week and daily. Smoking habits (categories: non-smoker, former smoker, and current smoker) were based on self-report. For daily alcohol consumption, the following categories were considered: less than 25g; 25 to 50g; 50 to 75g; 75 to 100g and over 100g. Gender, education (number of years of formal education), physical activity, smoking habits and alcohol consumption were used as control in the analysis. Briefly, we controlled for gender because female gender has been associated with a more depressive mood [34] and with higher levels of relative body fat [35]; education influence mood [30] and adiposity [36], physical activity impact on mood [31] and body adiposity [37] and the same is observed for smoking and alcohol consumption [37,38].

Anthropometric measures included weight (Tanita® BF 350 Body Composition Analyzer; Tanita Corporation, Tokyo, Japan), height (stadiometer Seca® 217; Seca GmBH & Co Kg, Hamburg, Germany) and WC (Seca® 201; Seca GmBH & Co Kg, Hamburg, Germany). BMI was calculated as weight (Kg)/height $(m)^2$.

Magnetic resonance imaging (MRI) acquisitions were performed to assess subcutaneous adipose tissue (SAT) and VAT using a 1.5T clinical approved Siemens Magnetom Avanto scanner. Centering the volumes between L3 and L5, T2-weighted true fast imaging with steady state precession images (TRUFI) were acquired using the following characteristics: repetition time (TR) = 4.69 ms, echo time (TE) = 2.06 ms, 6 axial slices, acquisition matrix = 256x256, field of view = 400 mm, in-plane resolution = 2.0 x 1.6 mm, slice thickness = 6 mm and gap between slices of 12 mm. The images were analyzed using OsiriX image processing software (OsiriX version 4.1.2, Pixmeo, Geneva, Switzerland). Three slices from each participant were selected and used: one slice centered in the L4 vertebra and, relative to that, an upper and a lower slice. In each slice, SAT and VAT were outlined manually and the respective areas calculated (Figure 1B). The mean values of each adiposity compartment (SAT and VAT) were calculated using data from the three slices and used as variables of interest for statistical analysis.

From the initial *n* = 120 participants, 10 did not perform the MRI acquisition due to MRI specific contraindications (claustrophobia, pacemakers and/or implanted metallic prostheses) and from the ones that performed the MRI acquisition 14 were excluded due to unsuitable MRI acquisition (excess of movement during the acquisition). Thus, the sample for the analysis regarding MRI adiposity measures comprised *n* = 96 subjects (Figure 1A).

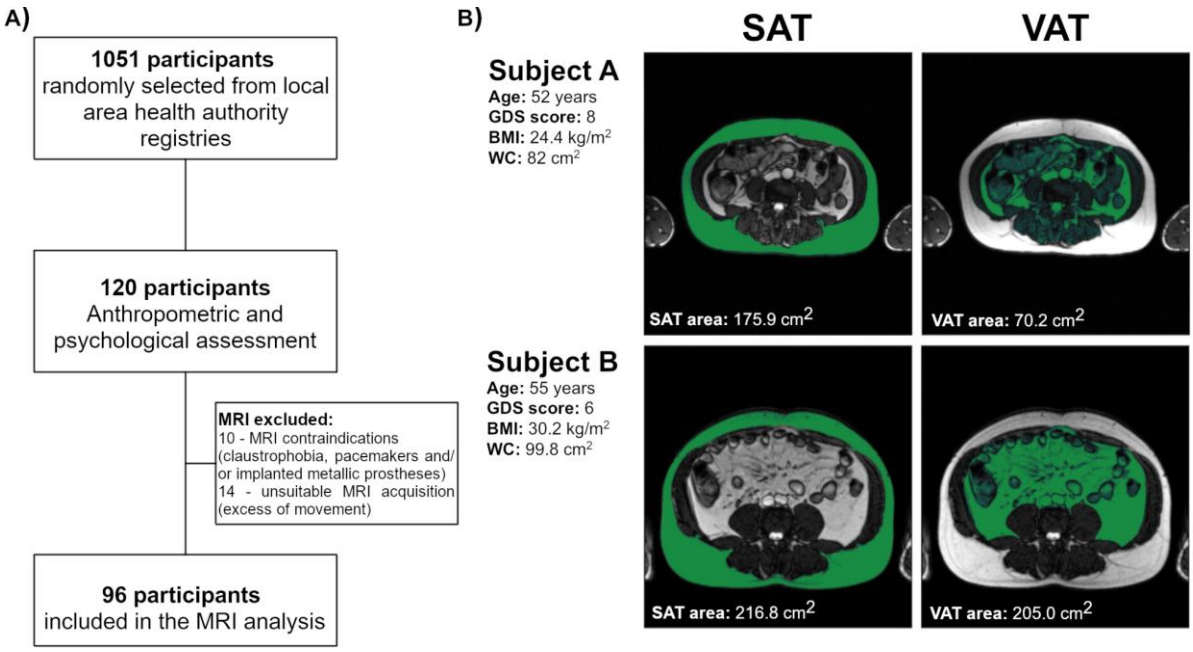

Figure 1 - Overview of study design. Flow diagram of participant's recruitment and selection **(1A)**. Representative images of the manual outline of SAT and VAT in a normal weight participant (subject A) and obese participant (subject B) **(1B)**.

Statistical analysis

Participants' characteristics are presented in mean and standard deviation (mean; SD). Pearson correlations coefficients were obtained to identify significant relationships between mood (GDS) and the variables from adiposity measures and indexes. Multiple Linear Regression Models (MLRM) were conducted to examine the predictive value of age and adiposity indexes/measures on GDS, adjusted for gender, education (school years), occupational status, physical activity, smoking and alcohol consumption. A moderation analysis was performed by testing the significance of the interactions of age and adiposity indexes, adjusted for the variables above mentioned, using MODPROBE release 2.0 for IBM SPSS Statistics v24 [39]. A statistically significant interaction indicates that the moderator variable (age) is

able to change the strength or trend of the association between the dependent variable (GDS score) and the independent variable (adiposity index). To allow the visualization of the moderation effect, *pick-a-point* plots were obtained using the syntax provided on MODPROBE output at mean and mean ± 1 SD. Values for the null effect of age and adiposity indexes were calculated using the regression equation. Statistical analysis was conducted using the IBM SPSS Statistics v24 and statistical significance was defined at $p < .05$ level.

RESULTS

Sample characteristics for the 120 participants regarding all measures are shown in Table 1, results considering the 96 participants eligible for the analysis using MRI data are presented in Supplementary Table 1. Our sample was distributed between males and females (females n=57; 47.5%), with a low education level which is representative of the Portuguese population in these age strata [31]. Age was positively correlated with VAT and WC (r_{WC}=.206; r_{VAT}=.226) which is in accordance with the increase of abdominal fat with age. BMI was positively correlated with the GDS score (r_{BMI}=.236) indicating that higher BMI was associated with a more depressive mood (Supplementary Table 2).

To test whether these associations remained when controlled for the main confounders, MLRM was used; the results are presented in Figure 2 and in Supplementary Table S3. All models are statistical significant and the set of independent variables explain 20% to 26% (R^2_{adj}=.195 to 0.263) of the GDS score. Age was negatively associated with GDS score in the model that includes VAT (β_{Age}=-.242) indicating that, when considering VAT, with an increase in age a decrease in GDS score is observed. In the respective models, BMI (β_{BMI}=.205), WC (β_{WC}=.193) and VAT (β_{VAT}=.283) were significant positive predictors of the GDS score, meaning that higher scores in the GDS were observed at higher adiposity levels. On the other hand, SAT was not a significant predictor of the GDS score (β_{SAT}=.048).

Subsequently, to test whether the association of adiposity with GDS score was dependent on age, a moderation analysis was performed (Figure 3 and Supplementary Table 3). All models are statistically significant and the set of independent variables explain 22% to 28% (R^2_{adj}=.224 to .282) of the GDS score. Significant BMI*age (β=-.190), WC*age (β=-.191) and SAT*age (β=-.212) interactions were observed. Of notice, VAT was a significant predictor of GDS without a significant VAT*age interaction effect while SAT*age interaction effect was significant without a significant effect of SAT. In these models, age presented a significant negative association with GDS while BMI, WC and VAT presented a positive

association with GDS. Figure 3 displays the representative *Pick-a-point* plots of the associations between GDS score and adiposity measures/indexes at mean age minus 1SD, mean age and mean age plus 1SD (Figure 3A, 3C, 3E and 3G) and the conceptual models of the associations (Figure 3B, 3D, 3F and 3H). In the plots representing BMI*age, WC*age and SAT*age interactions (Figure 3A, 3C and 3E) we can observe that, in younger subjects a higher adiposity measured was associated with a more depressive mood while in the older participants the adiposity presented a null or negative association with GDS score. The plot representing VAT*age interaction (Figure 3G) suggests that a higher VAT is associated with a higher GDS score independently of age.

Table 3 - Study sample characterization.

Characterization	
GDS score (mean, SD)	10.82; 6.48
Age (years) (mean, SD)	65.94; 8.42
Education (school years) (mean, SD)	5.16; 3.65
Gender (n, %)	
Females	57; 47.5
Males	63; 52.5
Physical activity (n, %)	
Sedentary	82; 68.33
Less than 3 times/week	17; 14.17
Over 3 times/week	12; 10
Daily	9; 7.5
Smoking (n, %)	
Non-Smoker	77; 64.17
Former-Smoker	31; 25.83
Smoker	12; 10
Alcohol consumption (n, %)	
<25g	59; 49.17
25-50g	29; 24.17
50-75g	11; 9.17
75-100g	16; 13.33
>100g	5; 4.17
Anthropometry (mean, SD)	
Weight (kg)	73.34; 12.26
Height (m)	1.58; 0.09
BMI (Kg/m^2)	29.15; 3.76
WC (cm)	96.12; 9.96
SAT (cm^2) †	213.88; 66.58
VAT (cm^2) †	150.31; 67.99

† n=96. GDS, Geriatric Depression Scale; BMI, Body Mass Index; WC, Waist Circumference; SAT, Subcutaneous adipose tissue; VAT, Visceral adipose tissue

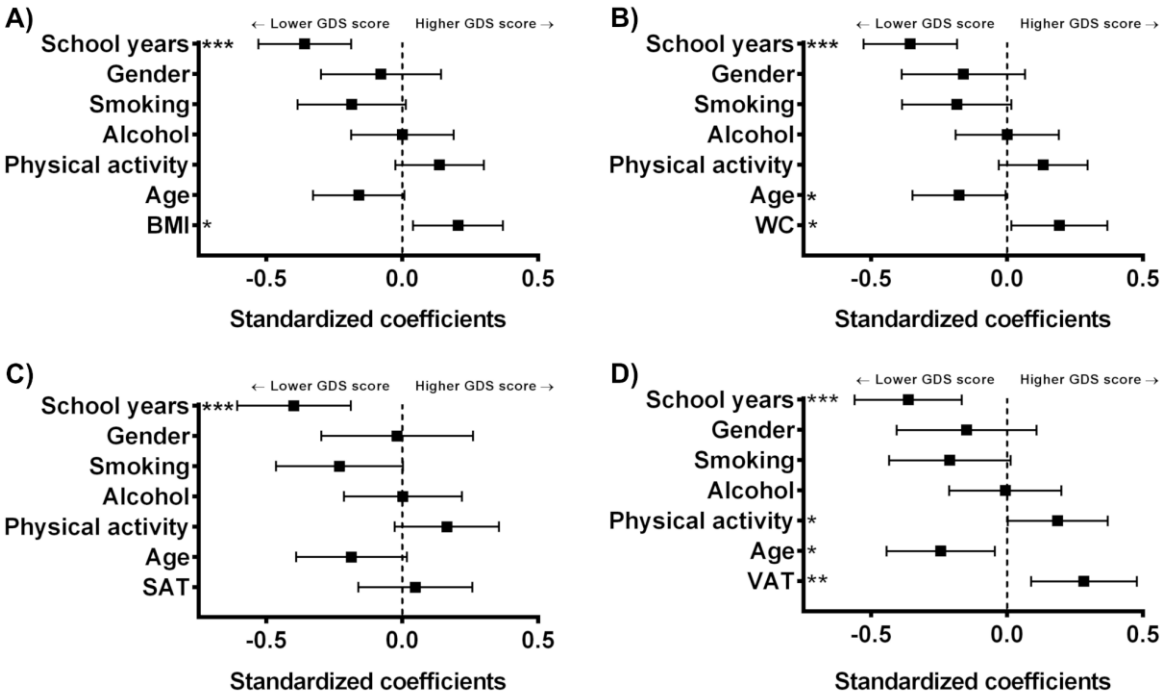

Figure 2 - Graphical representation of standardized coefficients (beta scores) and respective confidence intervals for the independent variables used in the multiple regression linear models. Higher formal education (measured in school years) and lower BMI are significantly associated with a lower score in the GDS **(2A)**. Higher formal education, older age and lower WC are significantly associated with a lower score in the GDS **(2B)**. Higher formal education is significantly associated with a lower score in the GDS **(2C)**. Higher formal education, lower physical activity frequency, older age and lower VAT area are significantly associated with a lower score in the GDS **(2D)**. Gender (0=females; 1=males), smoking (0=non-smoker; 1=former-smoker; 2=smoker), alcohol consumption (0=<25g; 1=25-50g; 2=50-75g; 3=75-100g; 4=>100g) and physical activity (0=sedentary; 1= less than 3 times/week; 2=over 3 times/week; 3=daily). * $p<.05$; ** $p<.01$; *** $p<.001$.

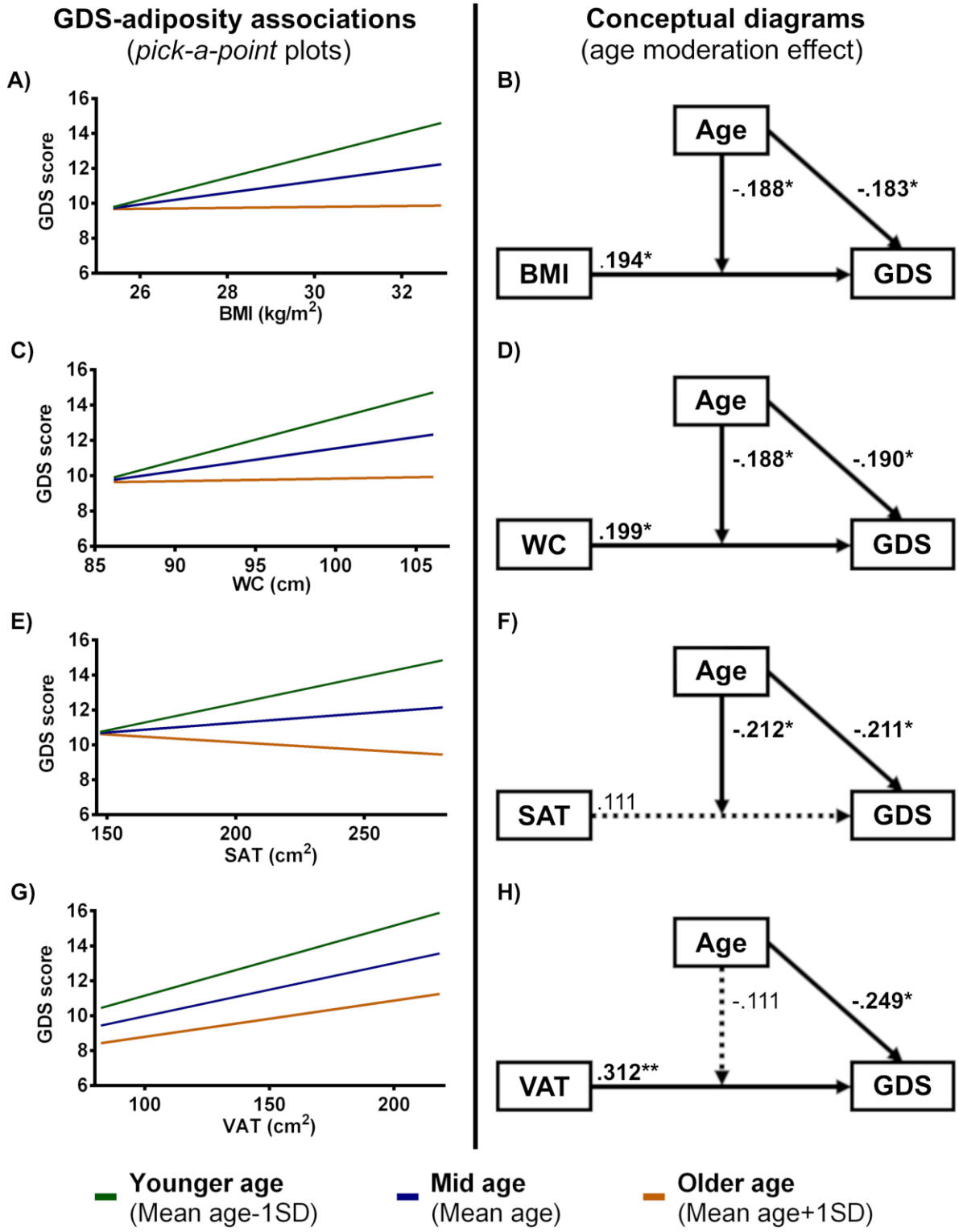

Figure 3 - Graphical representation of the moderation effect of age in the association between GDS score and adiposity measures. *Pick-a-point* plot of the association between GDS score and BMI at mean age and mean age ±1SD **(3A)**. Conceptual diagram of the effect of age on the association between GDS score and BMI **(3B)**. *Pick-a-point* plot of the association between GDS score

and WC at mean age and mean age ±1SD **(3C)**. Conceptual diagram of the effect of age on the association between GDS score and WC **(3D)**. *Pick-a-point* plot of the association between GDS score and SAT at mean age and mean age ±1SD **(3E)**. Conceptual diagram of the effect of age on the association between GDS score and SAT **(3F)**. *Pick-a-point* plot of the association between GDS score and VAT at mean age and mean age ±1SD **(3G)**. Conceptual diagram of the effect of age on the association between GDS score and VAT **(3F)**. * $p<.05$; ** $p<.01$.

Table S4 presents the age and adiposity indexes values with null effects on the association with GDS score for the interactions with significant moderator effects. The association between the respective adiposity index and GDS changes from positive to negative at the age interval between 70 and 79 years. In the same line, we present the values of adiposity indexes at which, independently of the age, no effect of adiposity on the GDS score is observed.

DISCUSSION

The present study indicates that, in older individuals (50+ years of age), the cross-sectional trend of the association between some adiposity indexes and mood depends on age. The study adds to the literature by exploring multiple adiposity measures, including the abdominal body fat compartments with MRI analysis. Here, BMI, WC and SAT were positively associated with GDS in the younger participants of our sample and the association became null or negative for the older ones. Of interest, VAT was positively associated with GDS independently of age.

The existing literature on this field has focused on the linear [14,20,21,40,41] or quadratic (U-shaped) [13] associations of adiposity and mood using age as a control variable. Positive associations between depressive symptomatology and adiposity measures, such as BMI [20,40], WC [20,21,40,42] and VAT [22,41], were found in studies focusing in middle age and older adults (≥45 years of age). In geriatric populations, other studies found that individuals with a higher BMI were less likely to suffer from depressive symptoms [15-19]. The disparity of results, the heterogeneity of the age of participants and the different measures of adiposity used, highlighted the need of studies addressing the effect of age as a moderator, as reported here. Two meta-analysis [12,43], mostly including studies in non-aging cohorts, have already suggested the moderator effect of age in the association of adiposity and mood. Since those meta-analyses included mostly studies

from non-aging cohorts, the absence of a moderator effect of age was explained as a result of the low number of studies that included elderly individuals.

To further understand the relevance of the present findings, it is important to discuss the biological processes underlying the association between adiposity and mood, which remain poorly understood [10,11]. Among the hypothesis put forward are inflammation and cytokine production [44], vascular alterations [45], insulin resistance [46] and dysregulation of the HPA-axis [47]. Furthermore, psychological factors are associated with overweight and obesity [48] since depressive mood can induce weight gain both by adaptation of unhealthy lifestyle [49] and/or dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis [50,51]. All of the above are suitable to explain the positive association observed in the younger individuals of our sample but fail to explain the change observed with the increase of age. Ho et al. [16], observed a negative association between BMI and depressive mood and hypothesized that a higher BMI could indicate greater physiologic and functional reserve (due to higher muscle mass) which protects against depressive mood. Moreover, low BMI can be a surrogate marker of chronic illness (illness-related weight loss) and it is well established that late-life depressive mood frequently occurs in the context of medical illness [52,53].

In the present study, VAT was a significant predictor of depressive mood. Yet, the association was not moderated by age. In accordance, previous reports show that depressive symptoms are associated with VAT in middle age women [22,41] and in older men [54]. According to the lipid overflow–ectopic fat model [55] the accumulation of adipose tissue in the visceral compartment can be the result of insulin resistance, which is associated with depressive mood [46], but also exacerbated by a neuroendocrine profile related to a maladaptive response to stress, which is also associated with depressive mood [56]. Even though both adipose compartments are associated with metabolic complications, it was demonstrated that VAT is a pathogenic fat depot [57], which is associated with systemic inflammation and cytokine production [58]. Furthermore, VAT has been recognized to be a major correlate of the cluster of metabolic complications that compose the metabolic syndrome [59] that can be associated with depressive symptomatology [11,45,60-63].

In line with our results, studies that tested the association of SAT with mood in middle-age subjects [22,25] and older subjects [26] found no significant association. Here we report for the first time that, in aged individuals, age moderates the association of SAT and depressive mood. In contrast with VAT, SAT has been hypothesized as a protective fat depot [64], whose decrease due to "anorexia of aging" and/or the

"illness-related weight loss" can explain the moderation effect here observed. Particularly, these results raise attention for the need to assess regional adiposity instead of a total body fat. Recently it has been proposed that higher adiposity levels can be protective in the elderly [65]. The data reported in the present study supports the notion that higher subcutaneous adiposity levels are associated with lower depressive mood in older individuals.

In the present work, we use indirect methods to assess adiposity, such as BMI and WC, but also direct and reliable methods such as MRI to obtain an accurate picture and to overcome the limitations inherent to each adiposity measure. This study tested associations with depressive symptomatology and not with clinical depression, which can mask the magnitude of the associations, reducing the interaction effect. In addition, GDS is a self-reported measure; it is therefore possible that participants try to conceal psychological issues (the same is true for the other self-reported measures, such as alcohol consumption, smoking habits and physical activity). Despite of these possible limitations, we used validated and widely used methods to minimize the risk of reporting bias. Furthermore, the cross-sectional nature of this work prevented us from drawing cause-effect conclusions.

In conclusion, this study provides evidence that the association of adiposity with depressive mood can be moderated by age. From the clinical perspective, this observation highlights the importance of assessing body composition in depressed patients and depressive mood in obese patients, paving the way for personalized therapeutic interventions that take into account these complex interactions.

Conflict of interest declaration

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgments

This work was funded by the European Commission (FP7): "SwitchBox" (Contract HEALTH-F2-2010-259772), and co-financed by project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER), by the Fundação Calouste Gulbenkian (Portugal) (Contract grant number: P-139977; project "Better mental health during ageing based on temporal prediction of individual brain ageing trajectories (TEMPO)") and by the Portuguese Foundation for Science and Technology (FCT) under the project PTDC/DTP-PIC/6936/2014. CPN was supported by the Portuguese Science Foundation (FCT) doctoral scholarship PD/BD/106050/2015 via Inter-University PhD Programme in Ageing and Chronic Diseases, TCC was a recipient of a doctoral fellowship from the Portuguese Foundation for Science and Technology (FCT; SFRH/BD/90078/2012), PSM was supported by the FCT fellowship grant with the reference PDE/BDE/113601/2015 from the PhDiHES program, PM was supported by the Fundação Calouste Gulbenkian (Portugal) Grant Number: P-139977: 'Better mental health during ageing based on temporal prediction of individual brain ageing trajectories (TEMPO)'. RM was supported by the FCT fellowship grant with the reference PDE/BDE/113604/2015 from the PhDiHES program. NCS was a recipient of a Research Assistantship by the through the FCT Investigator Programme 200∞Ciência.

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SUPPLEMENTARY MATERIAL

Supplementary Table 2 - Characterization of the 96 participants that were included in the body fat compartment analysis using magnetic resonance imaging.

Characteristics	**n=96**
GDS score (mean, SD)	11.35; 6.6
Age (years) (mean, SD)	65.12; 8.34
Education (school years) (mean, SD)	5.01; 3.5
Gender (n, %)	
Females	49; 51.04
Males	47; 48.96
Physical activity (n, %)	
Sedentary	67; 69.79
Less than 3 times/week	13; 13.54
Over 3 times/week	11; 11.46
Daily	5; 5.21
Smoking (n, %)	
Non-Smoker	63; 65.63
Former-Smoker	22; 22.92
Smoker	11; 11.46
Alcohol consumption (n, %)	
<25g	51; 53.13
25-50g	21; 21.88
50-75g	7; 7.29
75-100g	12; 12.5
>100g	5; 5.21
Anthropometry (mean, SD)	
Weight (kg)	72.23; 11.39
Height (m)	1.58; 0.09
BMI (Kg/m^2)	28.95; 3.57
WC (cm)	95.06; 9.6
SAT (cm^2)	—
VAT (cm^2)	—

Supplementary Table 2. Bivariate correlations of age, GDS score and adiposity.

	Age	**GDS**	**BMI**	**WC**	**SAT**
GDS	-.007				
BMI	.146	.236***			
WC	.206*	.086	.803***		
SAT	.043	.109	.711***	.489***	
VAT	.226*	.169	.588***	.763***	.174

BMI, Body Mass Index; WC, Waist Circumference; SAT, Subcutaneous Adipose Tissue; VAT, Visceral adipose tissue. *p<.05level; **p<.01; ***p<.001.

Supplementary Table 3. Linear regression and moderation analysis of age and adiposity indexes on mood.

	Linear regression analysis			Moderation analysis		
	B (CI 95%)	**SE**	**β; *p***	**B (CI 95%)**	**SE**	**β; *p***
Education	-.638 (-.941; -.335)	.153	-.359; <.001	-.627 (-.924; -.330)	.150	-.353; <.001
Gender	-1.017 (-3.871; 1.838)	1.441	-.079; .482	-1.443 (-4.263; 1.377)	1.423	-.112; .313
Smoking	-1.798 (-3.716; .121)	.968	-.187; .066	-1.787 (-3.668; .093)	.949	-.185; .062
Alcohol consumption	.003 (-.991; .998)	.502	.001; .995	.112 (-.867; 1.092)	.494	.021; .821
Physical activity	.935 (-.176; 2.047)	.561	.137; .098	.834 (-.258; 1.927)	.551	.122; .133
Age	-.123 (-.253; .006)	.065	-.160; .062	-.141 (-.268; -.013)	.064	-.183; .031
BMI	.354 (.069; .638)	.144	.205; .015	.334 (.054; .613)	.141	.194; .020
BMI*Age	—	—	—	-.036 (-.066; -.006)	.015	-.188; .020
$R^2_{adjusted}$; R^2	.248; .292			.277; .326		
$F_{(df1;df2)}$; *p*	$F_{(7; 112)}$=6.593; <.001			$F_{(8; 111)}$=6.709; <.001		
Education	-.633 (-.938; -.328)	.154	-.356; <.001	-.632 (-.930; -.333)	.151	-.356; <.001
Gender	-2.080 (-5.006; .846)	1.477	-.161; .162	-2.073 (-4.941; .794)	1.447	-.160; .155
Smoking	-1.786 (-3.720; .149)	.976	-.185; .070	-1.837 (-3.734; .060)	.957	-.191; .058
Alcohol consumption	.005 (-.999; 1.008)	.507	.001; .992	.049 (-.935; 1.034)	.497	.009; .921
Physical activity	.906 (-.212; 2.023)	.564	.133; .111	.769 (-.333; 1.87)	.556	.113; .169
Age	-.136 (-.268; -.004)	.067	-.177; .044	-.146 (-.276; -.016)	.065	-.190; .028
WC	.125 (.010; .240)	.058	.192; .033	.129 (.017; .242)	.057	.199; .025
WC*Age	—	—	—	-.013 (-.024; -.002)	.006	-.188; .019
$R^2_{adjusted}$; R^2	.238; .283			.269; .318		
$F_{(df1;df2)}$; *p*	$F_{(7; 112)}$=6.321; <.001			$F_{(8; 111)}$=6.461; <.001		
Education	-.754 (-1.149; -.358)	.199	-.399; <.001	-.781 (-1.168; -.394)	.195	-.414; <.001
Gender	-.248 (-3.924; 3.427)	1.850	-.019; .894	.263 (-3.360; 3.886)	1.823	.020; .886
Smoking	-2.2001 (-4.424; .022)	1.119	-.232; .052	-2.383 (-4.564; -.203)	1.097	-.251; .033
Alcohol consumption	.012 (-1.125; 1.150)	.572	.002; .983	.119 (-.997; 1.236)	.562	.023; .832
Physical activity	1.214 (-.204; 2.632)	.714	.164; .092	1.161 (-.227; 2.548)	.698	.157; .100
Age	-.148 (-.309; .013)	.081	-.187; .071	-.167 (-.325; -.009)	.080	-.211; .039
SAT	.005 (-.016; .026)	.010	.048; .650	.011 (-.010; .032)	.011	.111; .301
SAT*Age	—	—	—	-.002 (-.004; .000)	.001	-.212; .027
$R^2_{adj.}$; R^2	.204; .262			.239; .303		
$F_{(df1;df2)}$; *p*	$F_{(7; 88)}$=4.474; <.001			$F_{(8; 87)}$=4.723; <.001		
Education	-.688 (-1.060; -.316)	.187	-.364; <.001	-.675 (-1.048; -.303)	.187	-.358; .001
Gender	-1.965 (-5.344; 1.414)	1.700	-.150; .251	-2.111 (-5.497; 1.275)	1.704	-.161; .219
Smoking	-2.007 (-4.133; .119)	1.070	-.211; .064	-1.941 (-4.069; .186)	1.070	-.204; .073
Alcohol consumption	-.036 (-1.117; 1.045)	.544	-.007; .948	.029 (-1.057; 1.116)	.547	.006; .957
Physical activity	1.372 (.013; 2.730)	.684	.186; .048	1.473 (.103; 2.843)	.689	.200; .035
Age	-.194 (-.351; -.036)	.079	-.245; .016	-.197 (-.354; -.040)	.079	-.249; .015
VAT	.027 (.009; .046)	.010	.283; .005	.030 (.011; .050)	.010	.312; .003
VAT*Age	—	—	—	-.001 (-.003; .001)	.001	-.101; .277
$R^2_{adjusted}$; R^2	.271; .325			.272; .334		
$F_{(df1;df2)}$; *p*	$F_{(7; 88)}$=6.041; <.001			$F_{(8; 87)}$=5.448; <.001		

All the models were controlled for education (school years), gender (0=females; 1=males), smoking (0=non-smoker; 1=former-smoker; 2=smoker), alcohol consumption (0=<25g; 1=25-50g; 2=50-75g; 3=75-100g; 4=>100g) and physical activity (0=sedentary; 1= less than 3 times/week; 2=over 3 times/week; 3=daily).

Supplementary Table 4. Age and adiposity values with null effects the associations with significant moderation effects.

	Null effects	
	Age	**Adiposity**
BMI*Age	75 (years)	25.15 (kg/m^2)
WC*Age	76 (years)	84.58 (cm)
SAT*Age	71 (years)	130.88 (cm^2)
VAT*Age	—	—

CHAPTER III

Metabolic dysfunction and mood during later life: impact upon default mode network functional connectivity.

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(In preparation)

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ABSTRACT

Background: Numerous studies suggest that a relationship exist between depression and metabolic syndrome or components thereof. The degree to which the syndrome is expressed has been found to vary with age, potentially affecting the putative association. Functional imaging has demonstrated a possible association between functional connectivity in the default mode network (DMN-FC) and components of metabolic syndrome. In order to investigate their potential relationship, we performed a cross-sectional study, using confirmatory factor analysis (CFA) of the association between metabolic dysfunction and depressive mood as function of age. We then investigated how the manifest links might be related to DMN-FC.

Methods: A total of 1051 participants (50 years of age or greater) were randomly selected from area health registries and 943 were included in the cross-sectional analysis. Participants were assessed with respect to mood (Geriatric depression scale) and a metabolic evaluation was performed. From the original sample, 120 participants were selected for functional magnetic resonance imaging.

Results: Metabolic dysfunction was modeled as a second order latent variable using CFA. First order latent variables were obesity, glucose dysmetabolism, lipids imbalance and blood pressure. Using multiple linear regression models, we observed that metabolic dysfunction, glucose dysmetabolism and lipids imbalance were linearly associated with depressive mood, and the association with obesity was U-shaped. The association of metabolic dysfunction, obesity and glucose dysmetabolism with depressive mood is positive for the younger individuals in our sample and vanishes with aging. The FC of the right superior temporal gyrus with the DMN was found to be correlated both with obesity and depressive mood. In participants with higher obesity scores, FC increased with higher GDS scores, while in those with lower GDS scores, FC decreased. Age and blood pressure were associated with a more complex pattern of association between FC of the right supramarginal gyrus and GDS score.

Conclusion: Metabolic dysfunction is associated with depressive mood, and some components of metabolic dysfunction are associated in an age dependent manner. Furthermore, the association of mood and components of metabolic dysfunction are associated with differential patterns of DMN-FC. Combination of the effects of age, mood and metabolic dysfunction is likely to be fundamental to the explanation of the high heterogeneity found in the DMN-FC. Additional studies, both with larger sample size and longitudinal design, will be necessary to confirm the results we have presented.

Keywords: Metabolic Dysfunction, Mood, Age, Functional Connectivity, Default Mode Network.

INTRODUCTION

Depression is a highly prevalent mood disorder and A leading cause of mental health related disease, affecting an estimated 300 million people worldwide [1,2]. It is projected to become the leading cause of burden of disease by 2030 [3]. Symptoms of depression can be present even in the absence of formal criteria to diagnose major depression. Depressive symptoms are widespread in the elderly, with subthreshold depression being 2 to 3 times more prevalent than major depression. It is estimated that those older adults with depressive symptoms will develop major depression at a value of 8 to 10% per year. The consequences of depression in later life include increased health care use and attendant expenditure, as well as cognitive and physical decline similar to that observed in a variety of other medical and psychiatric conditions. Both major depression and even less severe depressive symptoms, have been associated with higher mortality and morbidity [4].

Metabolic syndrome (MetS) is a cluster of metabolic abnormalities associated with high risk of developing type 2 diabetes and/or cardiovascular disease. According to the International Diabetes Federation it is defined by the presence of visceral adiposity and at least two of the following criteria: hyperglycemia, dyslipidemia (high triglycerides and/or low HDL cholesterol) and hypertension [5]. Visceral obesity is a central component of MetS and the aging process is associated with an increased deposition of body fat in the abdominal region [6]. MetS component factors are more prevalent in older than younger adults. Age has been found to be associated with the way MetS is expressed and also with how different combinations of MetS components are associated with mortality risk [7]. While there is strong evidence for increased risk to individuals who arbor such metabolic abnormalities, it is unclear whether or not the association of these anomalies represents a single underlying pathogenic pathway [8].

Several cross-sectional studies have associated depression or depressive symptoms with various components of MetS. In meta-analyses of cross-sectional and longitudinal studies, obesity was found to be associated with depression [9,10]. In another meta-analytic review, which included subjects with type 1 or type 2 diabetes, depression was associated with poor glycemic control [11]. Furthermore, positive association between depressive symptoms and insulin resistance was found in a large community based study [12]. In a large cross-sectional study and in a prospective study of aged individuals low blood pressure was associated with depressive mood [13,14]. Findings regarding dyslipidemia have been inconsistent with positive, negative or null associations between total cholesterol and depressive mood having been reported. An association between lipid components and depressive mood has been hypothesized, but

published results are inconsistent [15]. Low HDL cholesterol, and a higher atherogenic index (total/HDL cholesterol and LDL/HDL cholesterol) was associated with major depression [16]. In one longitudinal analysis, a higher total to HDL cholesterol ratio was associated with a faster increase in depressive symptoms among women [15]. In other studies, higher triglyceride levels were observed in patients with ongoing major depression than in those in remission or controls [17,18].

It has been suggested that the association between MetS and depression is bidirectional. Pan *et al.* [19], in a meta-analysis that included 155,333, subjects demonstrated that MetS was associated with depression, that baseline MetS could predict the risk of developing depression, and that the reverse is also true. Analyses were influenced by MetS definitions (NCEP ATP-III *versus* IDF) and depression measures (diagnostic interview *versus* self-reported symptom scale).

Individuals diagnosed with MetS constitute a heterogeneous metabolic group, so presenting a significant obstacle to evaluating the association between MetS with depressive mood. Furthermore, the contribution of each of the components of the overall syndrome differs between individuals and the weight of the MetS components to the association with mood has yet to be determined. Confirmatory factor analysis has been used to construct a hierarchical four-factor model that represented MetS by insulin resistance, obesity, lipids and blood pressure, which may help to determine the contribution of each component to the overall syndrome [8,20,21].

Since MetS and its components are measures of peripheral metabolic dysfunction and depression is an abnormality of the central nervous system, it is important to explore the impact of peripheral metabolic alterations on brain function and connectivity. The default mode network (DMN) has been well studied, both in general and in the context of depressive symptomatology. There have been several reports of higher functional connectivity (FC) within the DMN, as well as between the DMN and other brain regions in patients with depression [22,23]. In contrast, age associated reduction in DMN-FC has been frequently reported in the population over 60 years of age [24,25]. Several lines of evidence suggest that individuals with metabolic disorders demonstrate alterations in DMN activity and FC [26] and that multiple factors, such as age, mood and metabolic abnormality may interact with one another to produce alterations on DMN-FC. To the best of our knowledge, these associations have not been fully elucidated in previous studies.

The association of MetS and its components with mood as a function of advancing age has not yet been studied using confirmatory factor analysis. Furthermore, the interaction of these factors with the DMN-FC needs to be further characterized. In the present study, we propose to (1) utilize the latent variable model

of MetS, referred to as metabolic dysfunction; (2) explore, in a cross-sectional investigation the potential association of metabolic dysfunction and its components with depressive mood in older individuals; (3) assess the impact of advancing age in the strength of those associations and (4) evaluate the impact of the interaction between metabolic dysfunction, age and mood upon DMN-FC.

SUBJECTS AND METHODS

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki (59th Amendment) and was approved by the Portuguese national ethical committee (Comissão Nacional de Protecção de Dados) and by relevant local ethics review boards (Hospital de Braga, Braga; Centro Hospitalar do Alto Ave, Guimarães). The study goals and the psychological and clinical assessments were explained to the participants, of whom all gave informed consent.

Study sample

Characterization of the cohort

The cohort was composed of 1051 participants randomly selected from two cities in the north of Portugal (Guimarães and Vizela) using the local area health authority registries as described elsewhere [27,28] (Figure 1). The cohort is representative of the Portuguese population with respect to gender (females, n = 560; 53.3%) and age (range: 50–97 years; M = 67.2, SD = 9.24). All the participants were local community-dwellers. Most were retired (n = 763, females 51.8%) and located in the medium socio-economic stratum of the Graffar scale (Class III; 61.6%, females 47.3%). For age and gender, the distribution of this database differs in less than 2% of that of the distribution for the Portuguese population, as estimated by the Portuguese authority on statistics (the "Instituto Nacional de Estatística") [29]. Exclusion criteria included the inability to understand informed consent, participant choice to withdraw from the study, inability to attend the clinical and neuropsychological assessment session(s), dementia and/or diagnosed neuropsychiatric and/or neurodegenerative disorder (medical records). A team of experienced clinicians performed a standardized clinical interview which also determined current medication use and, was

designed to detect and exclude disorders of the central nervous system (e.g. epilepsy and neurodegenerative disorders) as well as overt thyroid pathology [27,28]. As shown in Figure 1, 108 participants were excluded due to missing data, eaving a total of 943 participants to be included in the baseline analysis.

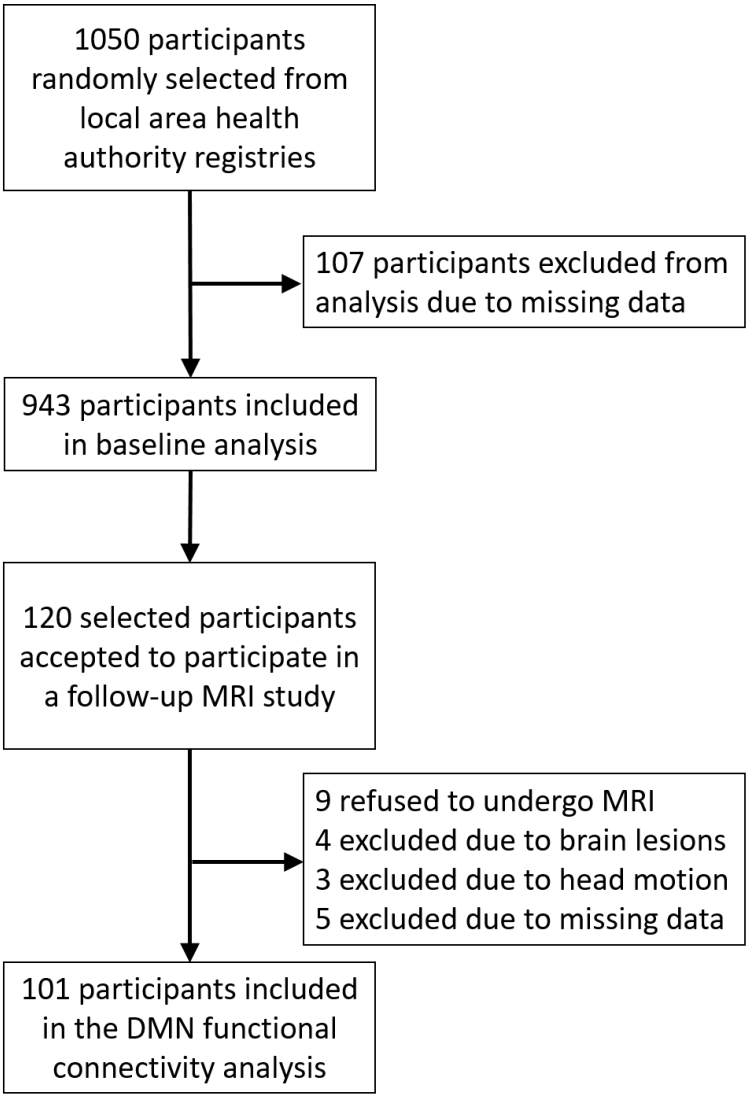

Figure 1 - Flow diagram of recruitment and study procedure.

From the initial cohort, 120 participants were selected for more comprehensive characterization, includng a functional magnetic resonance imaging (fMRI) session. Of those, 9 refused to undergo MRI, 4 were

excluded due to brain lesions, 3 were excluded due to excessive head motion during the scan and 5 were excluded due to missing data. Following exclusions, 101 participants ultimately were included in the fMRI analysis.

Metabolic and mood evaluation

Participants presented in the morning after overnight fasting and underwent a standardized evaluation that included medical history, anthropometric assessment, blood collection and blood pressure measurements. The anthropometric measures included weight (Kg), height (m), and abdominal perimeter (cm). Weight and height were subsequently used to calculate body mass index (BMI). Fasting blood glucose, fasting insulin, triglycerides and high-density lipoproteins were measured using standard methods. Blood pressure was assessed three times during the evaluation and the mean value was used. The Geriatric Depression Scale (GDS, long-version) was used to assess mood. Information regarding lifestyle variables is presented in the supplementary material.

MRI data acquisition, pre-processing and identification of DMN

Participants were scanned on a clinically approved Siemens Magnetom Avanto 1.5 T (Siemens Medical Solutions, Erlangen, Germany) MRI scanner in Hospital de Braga using a Siemens 12-channel receive-only head coil. During the resting-state fMRI acquisition, using gradient echo-weighted echo-planar images (EPIs), participants were instructed to keep their eyes closed and to attempt to think about nothing. The imaging parameters were: 180 volumes, TR = 2 s, TE = 30 ms, FA = 90°, in-plane resolution = 3.5 × 3.5 mm^2, 30 interleaved slices, slice thickness = 4 mm, imaging matrix 64 × 64 and FoV = 224 mm. T1-weighted structural images for anatomical reference were obtained using a magnetization-prepared rapid acquisition by gradient echo (MPRAGE) sequence with voxel resolution 1.0×1.0×1.0mm, Field of View (FOV) 234×234mm2, Flip Angle (FA) of 7°, 176 slices and Echo Time (TE)/Repetition Time (TR) of 3.48/2730ms. Before any data processing and analysis was undertaken, all acquisitions were inspected by a certified neuroradiologist who confirmed that they were not affected by critical head motion and that participants had no brain lesions.

Preprocessing of fMRI data was done using FMRIB Software Library (FSL v5.07) tools. The first five volumes of the acquisition were removed in order to exclude possible magnetic field inhomogeneities.

After this, the data underwent slice timing correction followed by head motion correction. Next, motion scrubbing [30] was performed in order to identify and further exclude time-points where head motion could be critical. At this step, one subject was excluded for having more than 15 motion-contaminated time-points. Each subjects functional dataset was then normalized to MNI standard space through a procedure that included: (i) skull stripping of the mean image of the functional acquisition and of the structural acquisition, allowing the isolation of brain signal; (ii) rigid-body registration of the mean functional image to the skull-stripped structural scan; (iii) affine registration of the structural scan to the MNI T1 template; (iv) nonlinear registration of the structural scan to the MNI T1 template using the affine transformation previously estimated as the initial alignment; (v) nonlinear transformation of the functional acquisition to MNI standard space trough the sequential application of the rigid-body transformation and nonlinear warp, followed by resampling to 2 mm isotropic voxel size. On the final step a linear regression of motion parameters, mean WM and CSF signal and motion outliers was performed and the residuals of the regression were smoothed using a Gaussian kernel smoother with a full width half maximum of 6 mm (σ = 2.55 mm), band-pass temporal filtered (0.01–0.08Hz) and then used for the subsequent analysis.

Probabilistic independent component analysis (PICA) was performed with MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components), distributed with FSL. ICA is a data driven analysis that isolates components or non-overlapping spatial maps corresponding to regions which manifest coherent time-courses. The software estimated group-wise spatial maps which correspond primarily to Resting State Networks (RSNs) and automatically estimated the number of independent components. In order to study subject-specific components a dual regression analysis was used.

Statistical Analysis

Data is presented in mean (M) and standard deviation (SD) for continuous variables and in frequency (n) and percentage (%) for categorical variables. Pearson correlations were calculated to measure the strength of the association between the quantitative variables studied. Structural equation models (SEM) were used to model metabolic dysfunction in a fashion similar to that reported by Shen *et al.* [8], using MPlus software version 7. The MLR estimator (maximum likelihood parameter estimates with standard errors and a chi-square test statistic that are robust to non-normality and non-independence of observations) was used for parameter estimations and tests of significance. Model fit was assessed using χ^2, comparative fit index (CFI), Tucker-Lewis index (TLI) and root mean square error of approximation

(RMSEA). To determine the cross-sectional associations of the GDS score with each of the composite scores previously calculated (metabolic dysfunction, obesity, glucose dysmetabolism, lipids imbalance and blood pressure) Multiple Linear Regression Models (MLRM) were performed controlling for age, gender, formal education, smoking habits, alcohol consumption, and physical activity. Besides regression coefficients (and confidence intervals), betas and measures of model fit (R^2, $R^2_{adjusted}$) are also presented. The interaction of age, and metabolic dysfunction (including components) with GDS score was tested using PROCESS 3.0 for IBM SPSS Statistics v25, adjusted for the variables above mentioned. Heat maps plots were obtained using the syntax provided on PROCESS output.

DMN-FC evaluation was performed using the second-level random effect analyses in SPM12. Multiple regression analysis was performed, and results were considered significant at $p < 0.001$, corrected for multiple comparisons using cluster correction (minimum cluster size of 70 voxels). Minimum cluster size was estimated using 3DClustSim (https://afni.nimh.nih.gov/; AFNI version 17.0.13; National Institute of Mental Health) with the DMN template mask and a significance level of 0.05. Anatomical labeling was performed by a combination of visual inspection and anatomical automatic labeling (AAL) atlas [31].

Several independent multiple regressions were performed to test the effect of metabolic dysfunction (or components), the interaction of metabolic dysfunction (or components) with age, the interaction of metabolic dysfunction (or components) with GDS score and the interaction of metabolic dysfunction (or components) with age and GDS score. The requirements for multiple linear regression analysis were met and the variables were mean centered to avoid multicollinearity issues. Age, gender and GDS score were used as covariables when not used as interest variables. For each participant, the mean Z score of the clusters with significant interactions were extracted and the values were used to generate heat maps which allowed visualization of the interaction of the moderator with the association between the focal predictor and the dependent variable.

RESULTS

Characterization of participants

Demographic, metabolic and lifestyle characterization of participants is presented in Table 1. Ages of participants ranged from 50 to 97 years (M=67 years and SD=9.17, 47.8% females) The exclusion of the 107 participants with missing data did not significantly change the composition of the original cohort.

Information on the prevalence of significant depressive symptoms and metabolic risk factors according the IDF classification for MetS [5] are described in supplementary material. Bivariate correlations between GDS score and metabolic parameters are presented in supplementary table 1. GDS score was significantly correlated with BMI (r=.106, $p<.01$), but not with the other parameters. Metabolic parameters were significantly associated among themselves, with the exception of systolic blood pressure with HDL-cholesterol, and diastolic blood pressure with fasting glucose and HDL-cholesterol.

Modeling of metabolic dysfunction and components

Metabolic dysfunction was modeled as a second order latent variable similar to the procedure used in *Shen et al.* [8,21] and *Levin et al.* [20] (Figure 2). All indicators were treated as continuous variables. First order latent variables (obesity, glucose dysmetabolism, lipids imbalance and blood pressure) were measured by their respective indicators. Specifically, obesity was measured by BMI and WC, glucose dysmetabolism by fasting glucose and HOMA-IR, triglycerides and HDL-cholesterol were the indicators for lipid imbalance and systolic and diastolic blood pressure were the measures of blood pressure. A higher order latent variable, metabolic dysfunction, was created with the first order latent variables enumerated above.

Three pairs of residual variances were correlated, one between BMI and triglycerides, a second between WC and triglycerides and a third between BMI and diastolic blood pressure. The incorporation of these correlations was justified by the known influence of obesity upon triglycerides and blood pressure.

Table 1 - Study sample characterization for the participants included in the cross-sectional analysis and fMRI analysis.

	Cross-sectional analysis	fMRI analysis
	n=943	n=101
Variable (M; SD)		
Age (years)	67; 9.17	64; 8.46
GDS score	10.92; 6.38	10.63; 6.63
BMI (kg/m^2)	28.42; 4.38	27.9; 3.62
Waist circumference (cm)	98.87; 10.42	97.4; 9.06
Fasting glucose (mg/dL)	94.88; 29.5	92.32; 30.13
HOMA2-IR	1.29; 1.2	1.36; 1.33
Triglycerides (mg/dL)	123.14; 70.09	132.96; 100.94
HDL (mg/dL)	54.51; 13.74	53.9; 13.6
Systolic BP (mmHg)	143.82; 19.76	141.95; 18.03
Diastolic BP (mmHg)	80.12; 10.3	81.56; 8.27
Gender (n; %)		
Female	492; 52.2	47; 46.5
Male	451; 47.8	54; 53.5
Formal education (n; %)		
4 years or less	792; 84	74; 73.3
More than 4 years	151; 16	27; 26.7
Smoking status (n; %)		
Nonsmoker	659; 69.9	66; 65.3
Former smoker	222; 23.5	26; 25.7
Smoker	62; 6.6	9; 8.9
Alcohol consumption (n; %)		
None	279; 29.6	30; 29.7
≤ 50 g/day	441; 46.8	41; 40.6
> 50 g/day	223; 23.6	30; 29.7
Physical activity (n; %)		
None	593; 62.9	68; 67.3
≤ 3 times/week	143; 15.2	12; 11.9
> 3 times/week	207; 21.9	21; 20.8

The fit of the model was confirmed by CFI = .981, TLI = .958 and RMSEA = .048. The model demonstrated that metabolic dysfunction could be summarized by 4 components that were defined by metabolic risk factors. Metabolic dysfunction was strongly a function of glucose dysmetabolism, moderately of lipid imbalance and obesity, and modestly of blood pressure. Stated differently, metabolic dysfunction explained 78% (R^2 = .781, p < .001) of glucose dysmetabolism, 52% of lipid imbalance (R^2 = .518, p < .001), 42% of obesity (R^2 = .417, p < .001) and 10% of blood pressure (R^2 = .103, p = .001).

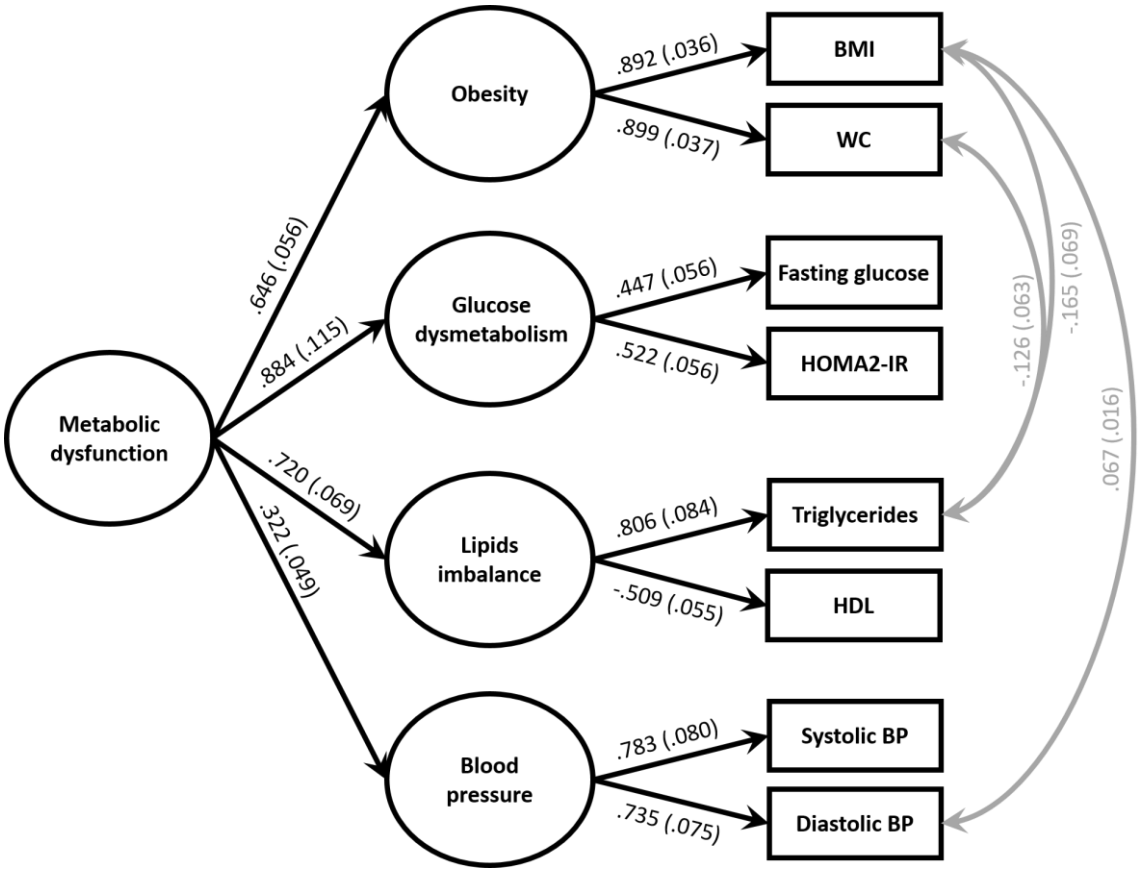

Figure 2 - Model of metabolic dysfunction at baseline. $\chi^2_{(13)}$=40.762; p<.001, comparative fit index (CFI)=.981, Tucker Lewis Index (TLI)=.958 and root mean square error approximation (RMSEA)=.048; p=.564. Standardized parameter estimates representing factor loadings are shown on paths. All coefficients are significant at p = 0.01 for the two-tailed test. To maintain presentation clarity, residual terms are not shown. BMI, body mass index; WC, waist circumference; HDL, high density lipoprotein; BP, blood pressure.

Associations of metabolic dysfunction and components with mood across later life.

To test the association of metabolic dysfunction and its components with depressive mood we use MLRM [Figure 3 (A, C, E, G and I) and Supplementary table 2]. GDS score was used as dependent variable, while metabolic dysfunction and its components as independent variable in different models, controlling for age, gender, education, smoking status, alcohol consumption and physical activity. All the models significantly predicted the GDS score (p<.001) and explained approximately 18% (R^2 = .179 to .185) of the variance of the GDS score.

In all the models, male gender, higher education (> 4 years of formal education), alcohol consumption and physical activity higher than 3 times per week were significantly associated with a lower GDS score.

In the respective models, metabolic dysfunction (β=.066, *p*=.029), glucose dysmetabolism (β=.062, *p*=.039) and lipids imbalance (β=.076, *p*=.011) were significantly associated with a higher GDS score. Furthermore, no linear association between depressive mood and obesity was observed, but a significant association between depressive mood and the quadratic term for Obesity ($Obsety^2$) was observed (β=.081, *p*=.007 – Supplementary table 3).

The effect of age on the association between metabolic dysfunction (or its components) and mood was tested through moderation analysis in MLRM [Figure 3 (B, D, F, H and J) and Supplementary table 2]. A significant moderation effect of age was observed in the association of GDS score with metabolic dysfunction (metabolic dysfunction * age - β=-.096, *p*=.047), obesity (obesity * age - β=-.065, *p*=.032) and glucose dysmetabolism (glucose dysmetabolism * age - β=-.066, *p*=.028). This moderating effect of age reflects a positive correlation between the measured variables in younger participants, but one which diminishes with advancing age. The association of the quadratic term of obesity with the GDS score (Supplementary table 3) was not influence by age.

The association between GDS score and the lipids imbalance component was not moderated by age (lipids imbalance * age - β=-.017, *p*=.592) indicating that lipids imbalance is positively associated with GDS score regardless of age. Also, no moderating effect of age was observed upon the association of blood pressure and GDS score (blood pressure * age - β=-.050, *p*=.098), indicating that blood pressure and mood are not significantly associated with one another at any age in the study population.

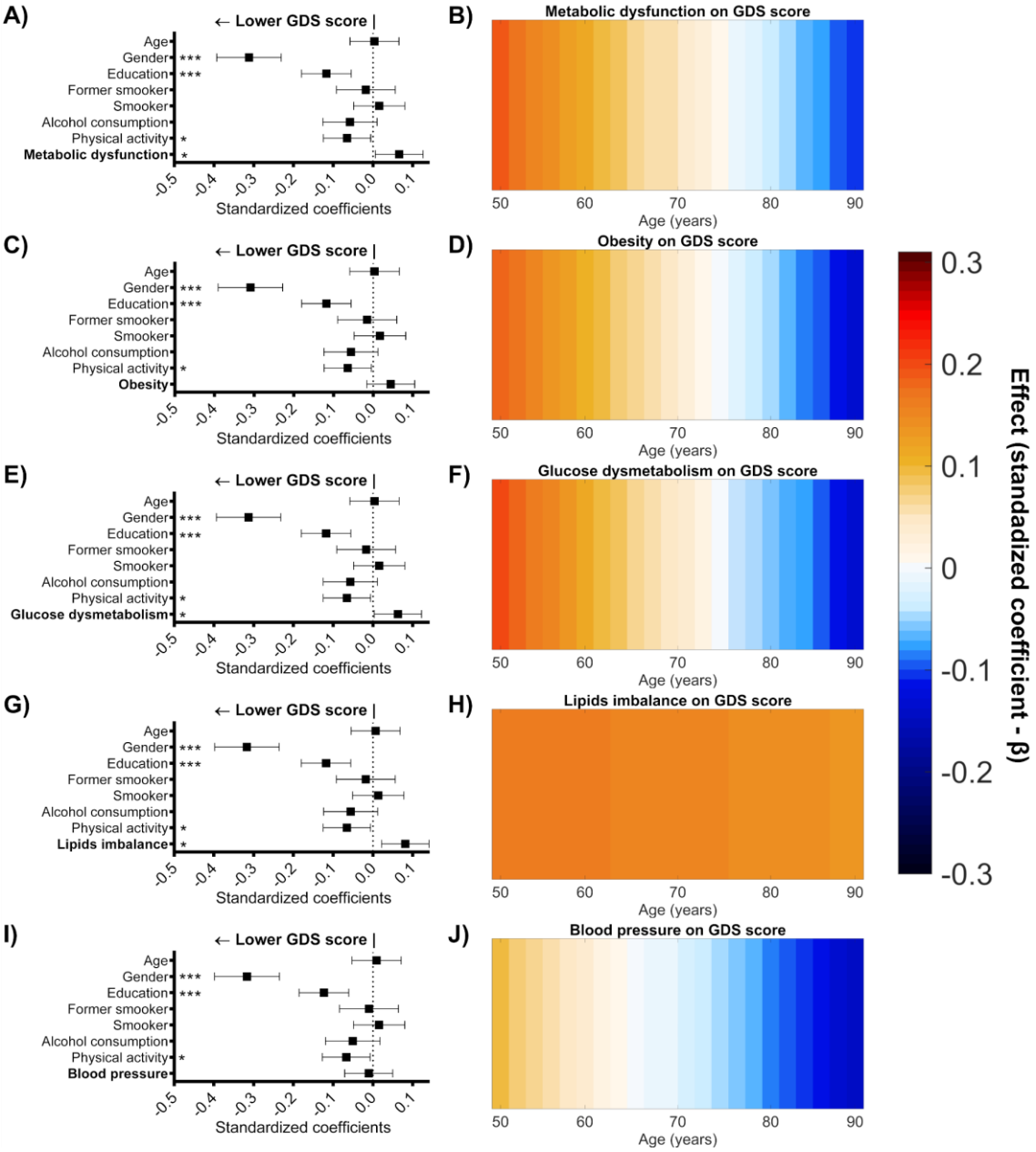

Figure 3 - Association of metabolic dysfunction (and components) with GDS score controlled for age, gender, education, smoking status, alcohol consumption and physical activity. Graphical representation of standardized coefficients (standardized beta) and respective confidence intervals for the independent variables used in the multiple regression linear models (2A, 2C, 2E, 2G and 2I). Male gender, higher formal education (measured in school years), higher physical activity and lower metabolic dysfunction score are significantly associated with a lower score in the GDS **(2A)**. Male gender, higher formal education and higher physical activity are significantly associated with a lower score in the GDS **(2C** and **2I)**. Male gender, higher formal education, higher physical activity and lower glucose dysmetabolism score are significantly associated

with a lower score in the GDS **(2E)**. Male gender, higher formal education (measured in school years), higher physical activity and lower lipids imbalance score are significantly associated with a lower score in the GDS **(2G)**. **Graphical representation (heat maps) of the variation of standardized coefficients (standardized beta) for the association between metabolic dysfunction (and components) with GDS score across age (2B, 2D, 2F, 2H and 2J).** Warm colors represent a positive association and cool colors represent a negative association. A positive association between GDS score and metabolic dysfunction or obesity or glucose dysmetabolism is observed in the younger individuals of our sample and becomes negative at the older ages **(2B, 2D and 2F).** The association between GDS score and lipids imbalance is always positive across the age range **(2H)**. No association between GDS score and blood pressure is observed across the age range **(2J)**. Gender (0=females; 1=males), smoking (reference - non-smoker), alcohol consumption (g/day) and physical activity (0=sedentary; 1= less than 3 times/week; 2=over 3 times/week). * $p<.05$; ** $p<.01$; *** $p<.001$.

Impact of metabolic dysfunction (and components) in the association between GDS score and DMN FC

To begin, the pattern of FC of the classical DMN regions during the resting state (Figure 4) were confirmed. The effects of metabolic dysfunction (or its components) and the interaction of age with metabolic dysfunction (or components) in the DMN-FC were evaluated, in independent MLRM, and the results did not survive to the significance threshold here employed.

Next, we tested the moderating effect of metabolic dysfunction (and its components) in the association between GDS score and DMN-FC. An interaction between obesity and GDS score was observed in the right superior temporal gyrus (Figure 5A and Table 2). In those participants having higher obesity scores, the FC of this area with the other components of the DMN increased with higher GDS score. Individuals with lower obesity scores, however, manifest decreased FC with increasing GDS (Figure 5B). No other interaction between metabolic dysfunction or its components with GDS was significant at the defined threshold.

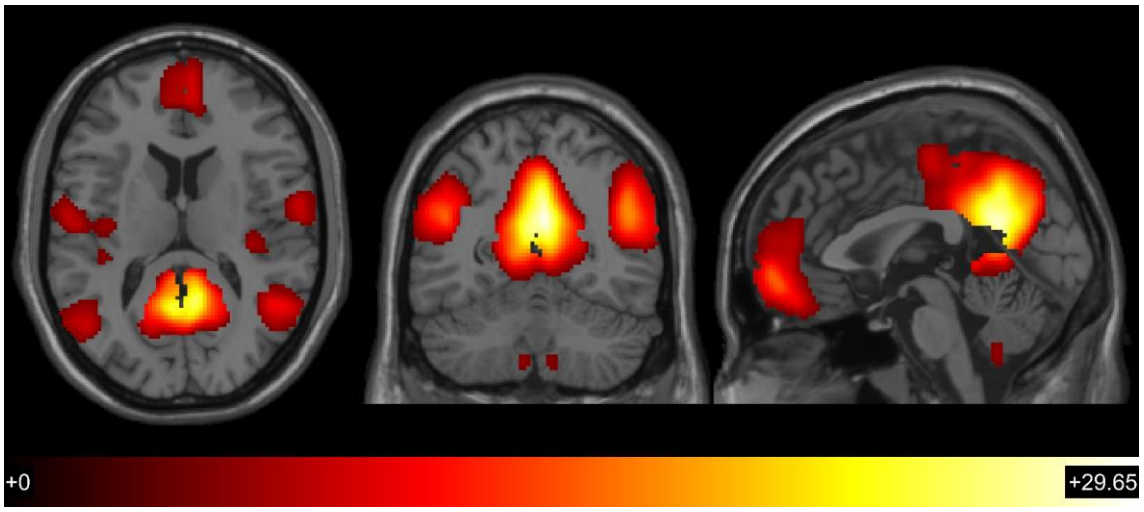

Figure 4 - Global patterns of default mode network functional connectivity.

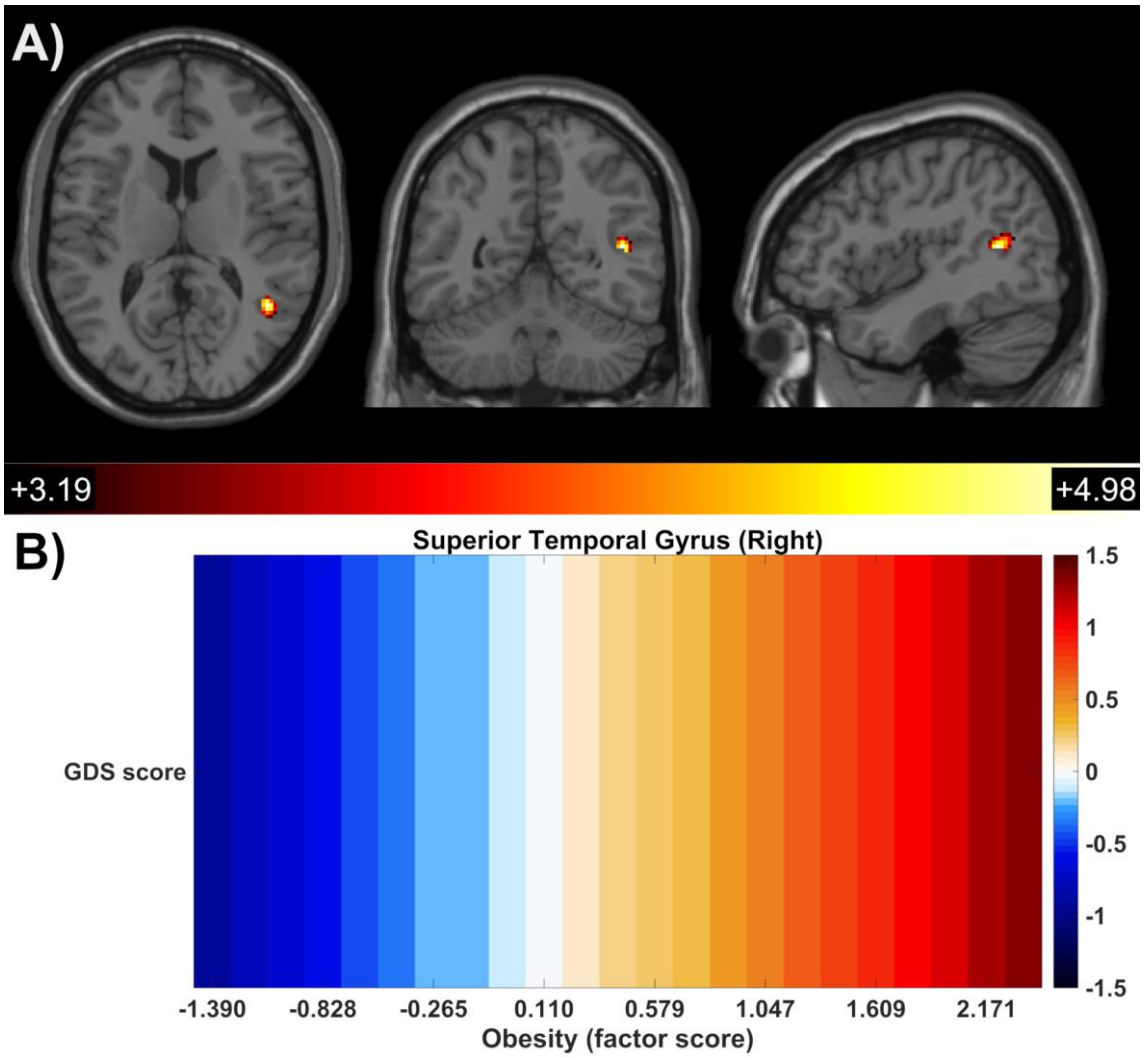

Figure 5 – Functional connectivity between a focal region of the right superior temporal gyrus and the other components of the DMN reflected GDS-obesity interaction. Colors represent standardized coefficients (β).

The moderating effect of age upon the interaction between metabolic dysfunction and GDS score with respect to DMN-FC

We also assessed the influence of age on the interaction of metabolic dysfunction with GDS score upon DMN-FC. A significant moderating effect of age was observed on the interaction of blood pressure with the GDS score in the level of FC of the right supramarginal gyrus with the other components of the DMN (Figure 6A and Table 2). For the younger participants in our sample (mean age – 1 SD), the FC of the right supramarginal gyrus with the DMN increased with higher GDS score in the participants which had lower blood pressure (mean blood pressure – 1 SD) (conditional effect, B=.021, p=.02), but was not significant for those subjects whose blood pressure was higher (mean blood pressure + 1 SD) (conditional effect, B=-.009, p=.359). Also, in the group of older participants (mean age + 1 SD), a decrease in the FC accompanied the GDS score increase in those who had lower blood pressure (mean blood pressure – 1 SD) (conditional effect, B=-.054, p<.001), while the opposite pattern was observed for those whose blood pressure was higher (mean blood pressure + 1 SD) (conditional effect, B=.025, p=.012) (Figure 6B). No other moderating effect of age upon the interactions of metabolic dysfunction and components with GDS score was statistically significant.

Discussion

In this study, we investigated the association of metabolic dysfunction and depressive mood in community-dwellers 50-years of age and older, and the relationship between those factors with FC of the DMN. We showed that depressive mood is linearly associated with metabolic dysfunction, glucose dysmetabolism and lipid imbalance. Conversely, the association of obesity with depressive symptomatology was found to be U-shaped. The influence of age upon the strength of the association of depressive mood with metabolic dysfunction, obesity and glucose dysmetabolism was substantial. We also observed that obesity can modify the association of GDS score with FC in the DMN, and that the interaction of age with blood pressure also affect the association of GDS score with the FC in the DMN.

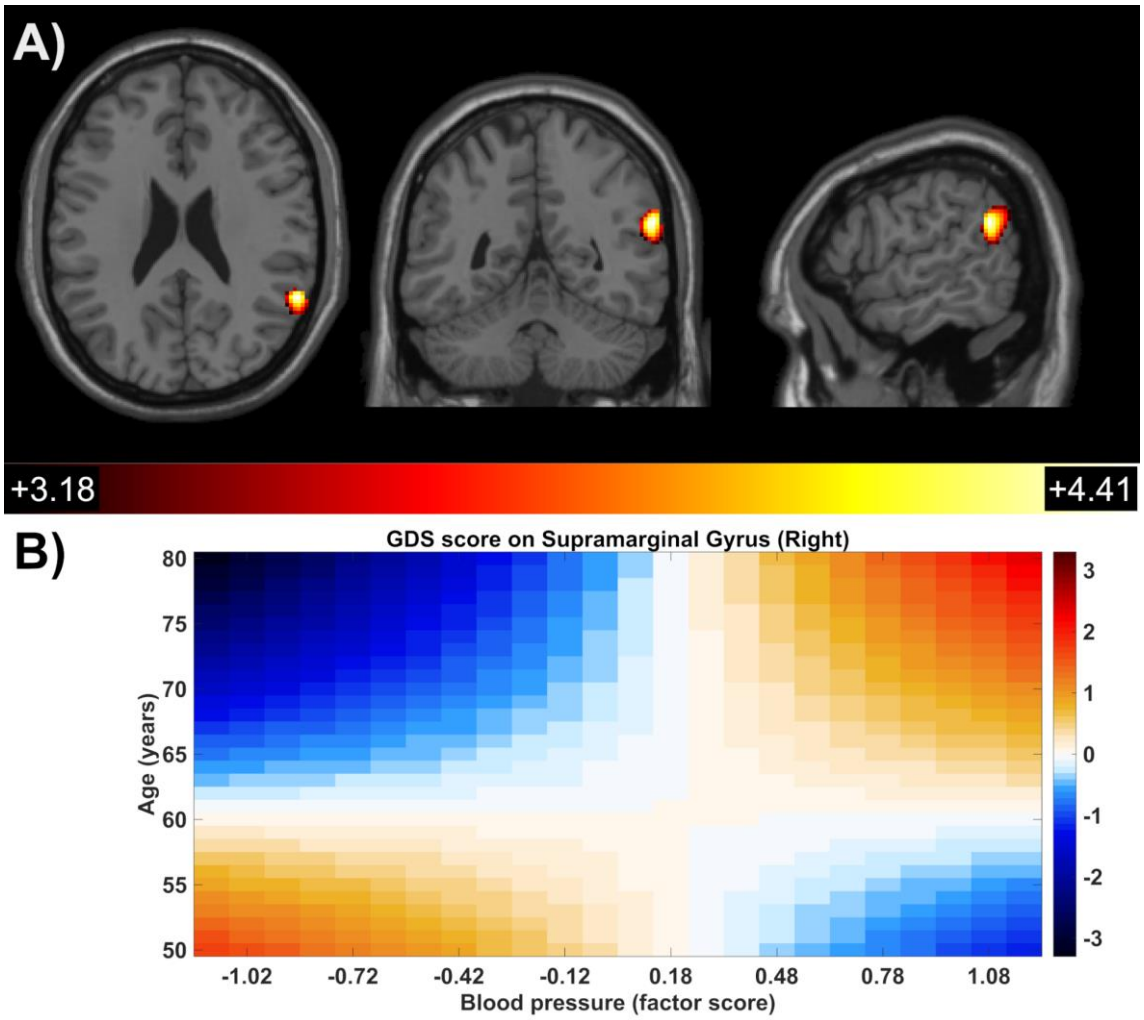

Figure 6 - Significant Age x GDS x Blood pressure interaction within the default mode network in the right supramarginal gyrus. Colors represent standardized coefficients (β).

Table 4 - Effect of the interaction between GDSxObesity and between AgexGDSxBlood pressure on the default mode network FC (multiple regressions, cluster correction, $p<.001$)

Effect	Region	Peak MNI coordinates	Cluster size (voxels)	Maximum Z score
GDS x Obesity	Superior Temporal Gyrus (Right)	44, -54, 12	81	4.98
Age x GDS x Blood pressure	Supramarginal Gyrus (Right)	60, -50, 24	234	4.19

Similar to the observations of others [8,21], glucose dysmetabolism appears to be the essential feature of metabolic dysfunction, a finding confirmed in both cross-sectional and longitudinal models. Insulin resistance is the most widely accepted hypothesis for the pathophysiology of the MetS [32] which would tend to support the large contribution of glucose dysmetabolism to metabolic dysfunction. The blood pressure component made a significant weaker contribution to metabolic dysfunction in all models. Shen *et al.* [8] reported a similar finding and argued that blood pressure may be related to metabolic dysfunction only secondarily.

As expected, we observed that metabolic dysfunction was associated with depressive mood. However, our analytic strategy does not allow inference about the direction of this association. In a meta-analysis, Pan *et al.* [19] observed that baseline depression predicts the risk of MetS and baseline MetS predicts the risk of depression, indicating a bidirectional association. MetS is not a clinical entity, but a cluster of risk factors which helps to identify individuals at risk of diabetes and cardiovascular disease [5,32,33]. Since MetS is a constellation of metabolic abnormalities, the association of MetS with depressive mood is likely to be mediated by its components. As previously stated, age plays an important role in moderating association between metabolic dysfunction and depressive mood. It was shown that the expression of MetS varies with age and that different combinations of MetS components are differentially associated with mortality risk [7]. It is also possible that the age variation in the expression of metabolic dysfunction influences its association with depressive mood.

There is strong evidence for a bidirectional association between obesity and mood disorders [10], but, we did not observe a linear association between obesity and GDS score. One possible explanation may be reflected in the U-shaped association between BMI and depressive mood [34]. In fact, we did observe a significant association of depressive mood with the quadratic term of the obesity factor, which is consistent with a U-shaped relationship. Accumulating evidence suggests that obesity and depression may mutually influence and reinforce one another. For instance, several studies have suggested that obesity may increase the risk of developing depressive symptoms, and *vice versa* [35,36]. Hypothalamic–pituitary–adrenal (HPA) axis activation occurs in stress and consequently in depression. It also is the cause the HPA axis is dysregulated in obesity and in those with MetS [35]. Chronic immune and inflammatory processes have been identified as accompanying features of obesity and are also seen with mood disorders [36]. Endocrine mediators, specifically adipokines, such as leptin and adiponectin, have also been associated with depressive mood. Leptin resistance and low levels of adiponectin appear to

increase the risk of depression. It also is the case that leptin resistance and low levels of adiponectin are common features of obesity [37]. The observed effect of age upon the association between depressive mood and obesity could potentially mask a linear association between them. In the present study, we observed that an association of obesity with depressive mood was positive for the younger participants and vanished with increasing age. While the mechanisms addressed above might explain the positive association observed in the younger participants, it fails to explain the loss of association seen in the older ages. Low BMI in older age can be a surrogate marker of chronic illness (illness-related weight loss) and it is well established that late-life depression frequently occurs in the context of medical illness [38,39]. Furthermore, it has previously been hypothesized that higher BMI could indicate a greater physiologic and functional reserve (due to higher muscle mass) which then protects against depressive mood in later life [40].

We have also observed a significant interaction between obesity and GDS score with the FC of the right superior temporal gyrus with the other identified components of DMN. The FC of the right superior temporal gyrus was positively associated with GDS score in those individuals with the higher obesity scores. Conversely, an opposite effect was observed for the individuals with lower obesity scores. In older adults, higher BMI has been associated with a decrease in FC of the posterior DMN [41]. DMN activity and connectivity has been demonstrated to be involved in one's own thoughts and feelings, self-referential thinking, recall the past and in planning for the future. One possible explanation for the hyperactivity and connectivity of DMN in patients with depression might be that it represents an inability to navigate away from their internal emotional states [22,23]. The results here presented are suggestive of a more resilient pattern of FC within the DMN in older individuals with low obesity score.

Another endocrine mediator associated with depressive mood is insulin resistance [11,42]. Data from epidemiological studies indicates that depression is twice as common among those with diabetes than in the general population, and that having diabetes doubles the risk of depression [43]. Furthermore, it seems that while the association is bidirectional, it is stronger in the direction of depression to type 2 diabetes [44]. Here, we observed a positive correlation between glucose dysmetabolism and depressive mood. Depression is associated with the activation of the HPA axis and production of pro-inflammatory cytokines which can induce insulin resistance [45,46]. Another hypothesis put forward to explain this association is that the inadequate glucose utilization that results from central insulin resistance is responsible for change at the neuronal level in vulnerable brain regions (e.g. limbic system) observed in patients with depressive disorders [47]. Data from animal models show that brain-specific knockout of insulin receptor (NIRKO) in

mice promotes age-related anxiety and depressive-like behavior through an alteration in dopamine turnover [48]. The relationship between depressive mood and glucose dysmetabolism was significantly moderated by age, similar to that which was observed for metabolic dysfunction and obesity. In younger participants the association was positive but lost strength with increasing age. A reasonable explanation for such a pattern is not evident, but we recognize that selection bias may be present. It is more likely that older individuals with higher levels of depression and higher comorbidities refused to participate in the study.

We also observed a significant positive association between depressive symptoms and lipid metabolism which was not moderated by age. Greater lipid imbalance factor is manifest in higher values of triglycerides and lower levels of HDL cholesterol. Research on the association between serum lipids and depression has generated conflicting results and has focused primarily on total cholesterol [15]. Inother studies, lower HDL cholesterol was reported to be associated with depression [49,50]. While high triglyceride levels were found in patients with bipolar depression when compared with healthy controls [50]. In persistent-severe depression the odds ratio for low HDL cholesterol and hypertriglyceridemia were significantly increased in men, and a similar association was observed for women with respect to hypertriglyceridemia [51]. Higher levels of triglycerides and lower HDL cholesterol in ongoing major depression, compared to remited depression and comtrols, also has been reported [17].

Blood pressure was the only component of metabolic dysfunction which was not linked to depressive mood and the absence of such an association was not a result of age moderation. The association of blood pressure with depression is controversial. Some cross-sectional studies reported an association between depression and low blood pressure [13,52], while others longitudinal studies found that depressive symptoms predicted low blood pressure [53] and that low blood pressure was a risk factor for higher levels of depression [14]. Other publications have reported a significant association of late life depression with hypertension [54]. While similar to our observations, others saw no association between blood pressure and depressive mood [51]. The lack of an association between depressive mood and blood pressure in the present study would appear to challenge the Vascular Depression hypothesis [55], which posits that cerebrovascular disease, of which hypertension is one element, may predispose, precipitate, or perpetuate certain geriatric depressive syndromes.

While no association between depressive mood and blood pressure, and no moderating effect of age in this association was demonstrable, a significant interaction between blood pressure, age and GDS score

was observed in the FC of the supramarginal gyrus. Recently Gu *et al.* [56] reported that, compared to controls, hypertensive patients with normal cognition manifested increased FC in the core subsystem of the DMN, including that of the right supramarginal gyrus. Furthermore, Zang *et al.* [57] found increased node centrality in drug-naive, first-episode major depressive disorder patients in components of the DMN, including the right supramarginal gyrus. Similarly decreased FC in the DMN has been widely reported in older subjects [25,58,59]. Collectively, these multiple studies suggest that the pattern of the FC of the right supramarginal gyrus is a complex one, influenced by multiple factors. One possible explanation for the negative association of FC and GDS score in older individuals with low blood pressure might be that it represents some form of protective effect.

To the best of our knowledge, this study is the first to evaluate the association of depressive mood with metabolic dysfunction and its components using confirmatory factor analysis. The age composition of the sample is representative of the Portuguese population and represents a strength of this study. A number of reports have addressed the association of MetS and depressive mood [19]. The use of the MetS classification has led to imprecise classification, such as including in the same group individuals with different metabolic profiles and differing levels of metabolic abnormality [7]. Another limitation, inherent to the use of the IDF classification for MetS, is that it excludes individuals who have metabolic risk factors but not central obesity [33]. To overcome those limitations we used structural equation modeling, more specifically confirmatory factor analysis, to replicate the hierarchical four-factor model first proposed by Shen *et al.* [8,21] and later replicated by Levin *et al.* [20]. The use of structural equation modeling allowed us to consider the separate contribution of each component to metabolic dysfunction. Additionally, it has the attribute of allowing one to work with continuous variables rather than employ a dichotomous classification which relies upon a proxy to assess severity of metabolic dysfunction. As a measure of depressive mood we used the GDS which measures depressive symptomatology rather than depression. The inclusion of subjects that have a finite GDS score but are not clearly depressed can weaken the association here observed, yet there is evidence that in older adults, even depressive symptoms are associated with adverse outcomes and morbidity [4]. The analysis of the moderating effects on the FC of the DMN adds to the analysis of the association between mood and metabolic dysfunction by exploring the central nervous system impact of peripheral mechanisms. The originality of this work is manifest in the use of structural equation models to explore the association of metabolic dysfunction with depressive mood, the impact of age upon those associations and in the combined influence of those multiple factors upon connectivity patterns in the DMN. Better understanding of the complex associations between metabolic dysfunction

and depressive mood will be dependent upon detailed analysis of the multiple interactions between the variables here studied.

In older individuals, our data demonstrate that metabolic dysfunction, obesity, glucose dysmetabolism and lipids imbalance are positively associated with depressive mood. For the parameter's metabolic dysfunction, obesity and glucose dysmetabolism the influence is significant for the younger members of the study sample, but not for older members. The association with lipids imbalance, however, unaffected by age. The fact that there is a demonstrable effect of several of the variables here studied upon the FC of at least one site within the DMN suggests that it is a central component to the mechanism between metabolic dysfunction and mood. Whether the modulation of FC is a cause of, or a consequence of the metabolic dysfunction has not been established, nor has the mechanism by which it may operate. Modifiable risk factors, such those which contribute to metabolic dysfunction, are potential targets for interventions designed to improve mood in later life. Understanding how peripheral factors interact, and how they might impact at the central mechanisms will be is paramount to the successful design of more effective strategies for prevention and treatment.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Contributions

CPN, JR, PSM, PC and JMB performed the statistical analysis. CPN, JR, PSM, TCC, LA, RM, PC and JMB contributed to the data analyses and discussion. NCS maintains the database and organized the neurocognitive/psychological sessions. PGC organized the evaluation sessions, and participant recruitment and collected the data. CPN, TCC and LA collected the data. AC, RM, PM and JMS collected and processed fMRI data. CPN wrote the first draft of the manuscript. JP, NS and JMB conceived and designed the study. All the authors revised the manuscript. NS had access to all the data in the study.

Acknowledgments

This work was funded by the European Commission (FP7) "SwitchBox" (Contract HEALTH-F2-2010-259772) project and co-financed by the Portuguese North Regional Operational Program (ON.2—O Novo Norte) under the National Strategic Reference Framework (QREN), through the European Regional Development Fund (FEDER), and by Fundação Calouste Gulbenkian—Inovar em Saúde ("Envelhecimento cognitivo saudável - proporcionar saúde mental no processo biológico do envelhecimento," Contract P-139977) and by the Portuguese Foundation for Science and Technology (FCT) under the project PTDC/DTP-PIC/6936/2014. C.P.N. was supported by the Portuguese Science Foundation (FCT) doctoral scholarship PD/BD/106050/2015 via Inter-University PhD Programme in Ageing and Chronic Diseases. J.R. was supported by the FCT fellowship grant with the reference PDE/BDE/113602/2015 from the PhDiHES program. A.C. was supported by a scholarship from the project NORTE-08-5639-FSE-000041 (NORTE 2020; UMINHO/BD/51/2017). P.S.M. was supported by the FCT fellowship grant with the reference PDE/BDE/113601/2015 from the PhDiHES program. T.C.C. was a recipient of a doctoral fellowship from the Portuguese Foundation for Science and Technology (FCT; SFRH/BD/90078/2012). R.M. was supported by the FCT fellowship grant with the reference PDE/BDE/113604/2015 from the PhDiHES program. P.M. was supported by the Fundação Calouste Gulbenkian (Portugal) grant number: P-139977: "Better mental health during ageing based on temporal prediction of individual brain ageing

trajectories (TEMPO).” L.A. was a recipient of a doctoral fellowship from the Portuguese Foundation for Science and Technology (FCT; SFRH/BD/101398/2014). N.C.S. was a recipient of a Research Assistantship through the FCT Investigator Programme 200∞Ciência.

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SUPPLEMENTARY MATERIAL

Methods – Information on lifestyle variables

For lifestyle, at baseline, alcohol consumption (none, 50 or less, and more than 50 grams/day), physical activity status (none, <3, and over 3 times per week), and smoking habits (non-smoker, former smoker, and current smoker) were considered. Alcohol consumption was calculated and recorded as total grams/day, taking as reference the estimations of grams of alcohol per glass for each consumed beverage. Physical activity included any planned activities (e.g., walking, jogging, swimming) that comprised a continuous 30 min effort (which could range from light, to moderate and vigorous) above the everyday living activities such as the case of regular short walk to the grocery store. Activity quantity rather than intensity was considered due to the mixed clinical profiles and age range of the study population. Alcohol consumption and smoking habits were self-reported by the participants during the clinical interview and were referent to the current habits.

Results – Additional information on sample characterization

Approximately half of our sample (n=449, 47.6%) presented some degree of depressive symptomatology (GDS score ≥11). Regarding BMI, 433 subjects (45.9%) presented overweight (25 $kg/m^2 \leq$ BMI < 30 kg/m^2), 300 (31.8%) were obese (BMI ≥ 30 kg/m^2) and only 210 participants (22.3%) had normal weight (BMI < 25 kg/m^2). The majority had an increased WC (n=781, 82.8%), that was more frequent in females (females WC > 80 cm - n=477, 97.7%; males WC > 94 cm – n=304, 67.4%) indicating a high risk of metabolic complications among those participants. Fasting plasma glucose was elevate in 245 participants (26%; fasting glucose≥100 mg/dL), raised triglycerides were present in 215 participants (22.8%; triglycerides>150 mg/dL), 223 (23.6%) participants had reduced HDL-cholesterol (HDL-cholesterol<50mg/dL in females and <40mg/dL in males) and elevated blood pressure was present in 702 participants (74.4%: systolic blood preassure≥130mmHg and/or diastolic blood preassure≥85 mmHg).

Supplementary table 1 – Correlation matrix among GDS score and metabolic parameters.

	GDS score	BMI	Waist circ.	Fasting glucose	HOMA2-IR	Triglycerides	HDL	Systolic BP	Diastolic BP
GDS score	4.700	2.966	4.048	2.631	.173	17.758	-.017	-7.606	.798
BMI	.106**	19.165	36.620	25.786	1.581	52.406	-11.734	1.530	8.536
Waist circ.	.061	.803***	108.509	67.196	3.446	152.989	-29.292	31.967	16.084
Fasting glucose	.014	.200***	.219***	87.047	8.241	498.476	-69.498	9.018	14.575
HOMA2-IR	.023	.302***	.276***	.233***	1.434	2.322	-3.490	2.072	.849
Triglycerides	.040	.171***	.210***	.241***	.242***	4913.080	-399.321	224.146	135.484
HDL	.000	-.195***	-.205***	-.171***	-.212***	-.414***	188.908	-3.264	-3.715
Systolic BP	-.060	.122***	.155***	.154***	.088**	.162***	-.012	39.304	116.095
Diastolic BP	.012	.189***	.150***	.048	.069*	.188***	-.026	.570***	106.104

*p < .05level; **p< .01; ***p< .001. Parameters' variances are represented on the diagonal of the table (light grey); parameters' covariances are represented on the upper-triangle (dark grey); correlation coefficients are represented on the lower triangle (no shading).

Supplementary table 2 - Linear regression metabolic dysfunction and components in mood and moderation analysis of age.

GDS score	B (CI 95%)	SE	β; *p*	B (CI 95%)	SE	β; *p*
Age	.002 (-.041; .046)	.022	.004;.911	.001 (-.042; .044)	.022	.001; .968
Gender [a]	**-3.988 (-5.022; -2.954)**	**.527**	**-.312; <.001**	**-4.072 (-5.108; -3.036)**	**.528**	**-.319; <.001**
Formal education [b]	**-2.041 (-3.123; -.960)**	**.551**	**-.117; <.001**	**-2.043 (-3.122; -.963)**	**.550**	**-.117; <.001**
Former smoker [c]	-.264 (-1.376; .849)	.567	-.018; .642	-.270 (-1.381; .84)	.566	-.018; .633
Smoker [c]	.408 (-1.254; 2.070)	.847	.016; .630	.471 (-1.19; 2.131)	.846	.018; .578
Alcohol consumption	-.010 (-.023; .002)	.006	-.058; .096	-.010 (-.022; .003)	.006	-.054; .120
Physical activity	**-.397 (-.759; -.035)**	**.184**	**-.065; .031**	**-.419 (-.78; -.057)**	**.184**	**-.069; .023**
Metabolic dysfunction	**.896 (.086; 1.706)**	**.413**	**.066; .030**	.804 (-.01; 1.618)	.415	.059; .053
Metabolic dysfunction X Age	–	–	–	**-.096 (-.19; -.001)**	**.048**	**-.060; .047**
$F_{(df1;df2)}$; R^2; $R^2_{adjusted}$; *p*	$22.884_{(8; 934)}$; .164; .157; <.001			$20.846_{(9; 933)}$; .167; .159; <.001		
Age	.002 (-.041; .046)	.022	.003; .913	.000 (-.044; .043)	.022	.000; .988
Gender [a]	**-3.95 (-4.991; -2.908)**	**.531**	**-.309; <.001**	**-4.002 (-5.043; -2.961)**	**.530**	**-.314; <.001**
Formal education [b]	**-2.053 (-3.138; -.967)**	**.553**	**-.118; <.001**	**-2.048 (-3.131; -.964)**	**.552**	**-.118; <.001**
Former smoker [c]	-.231 (-1.345; .883)	.568	-.015; .684	-.233 (-1.345; .879)	.567	-.015; .681
Smoker [c]	.437 (-1.228; 2.102)	.848	.017; .606	.585 (-1.082; 2.251)	.849	.023; .491
Alcohol consumption	-.010 (-.023; .002)	.006	-.056; .106	-.010 (-.022; .003)	.006	-.054; .121
Physical activity	**-.391 (-.754; -.028)**	**.185**	**-.064; .035**	**-.422 (-.785; -.059)**	**.185**	**-.069; .023**
Obesity	.329 (-.12; .778)	.229	.044; .151	.367 (-.083; .817)	.229	.050; .110
Obesity X Age	–	–	–	**-.056 (-.106; -.005)**	**.026**	**-.065; .032**
$F_{(df1;df2)}$; R^2; $R^2_{adjusted}$; *p*	$22.491_{(8; 934)}$; .162; .154; <.001			$20.584_{(9; 933)}$; .166; .158; <.001		
Age	.003 (-.040; .046)	.022	.004; .897	.001 (-.042; .044)	.022	.002; .958
Gender [a]	**-3.999 (-5.033; -2.965)**	**.527**	**-.313; <.001**	**-4.083 (-5.118; -3.048)**	**.527**	**-.320; <.001**
Formal education [b]	**-2.057 (-3.138; -.977)**	**.551**	**-.118; <.001**	**-2.045 (-3.123; -.966)**	**.550**	**-.118; <.001**
Former smoker [c]	-.252 (-1.365; .860)	.567	-.017; .656	-.267 (-1.378; .843)	.566	-.018; .636
Smoker [c]	.408 (-1.254; 2.071)	.847	.016; .630	.476 (-1.184; 2.136)	.846	.018; .574
Alcohol consumption	-.010 (-.023; .002)	.006	-.057; .101	-.010 (-.022; .003)	.006	-.053; .125
Physical activity	**-.400 (-.762; -.038)**	**.184**	**-.066; .030**	**-.423 (-.785; -.062)**	**.184**	**-.070; .022**
Glucose dysmetabolism	**1.149 (.057; 2.24)**	**.556**	**.063; .039**	1.045 (-.048; 2.138)	.557	.057; .061
Glucose dysmetabolism X Age	–	–	–	**-.143 (-.270; -.015)**	**.065**	**-.066; .028**
$F_{(df1;df2)}$; R^2; $R^2_{adjusted}$; *p*	$22.818_{(8; 934)}$; .163; .156; <.001			$20.902_{(9; 933)}$; .168; .160; <.001		
Age	.005 (-.038; .048)	.022	.007; .835	.004 (-.039; .047)	.022	.006; .843
Gender [a]	**-4.047 (-5.079; -3.015)**	**.526**	**-.317; <.001**	**-4.071 (-5.107; -3.035)**	**.528**	**-.319; <.001**
Formal education [b]	**-2.059 (-3.137; -.981)**	**.549**	**-.118; <.001**	**-2.072 (-3.152; -.993)**	**.550**	**-.119; <.001**
Former smoker [c]	-.272 (-1.382; .838)	.566	-.018; .631	-.270 (-1.38; .841)	.566	-.018; .634
Smoker [c]	.345 (-1.315; 2.005)	.846	.013; .684	.348 (-1.313; 2.009)	.846	.014; .681
Alcohol consumption	-.010 (-.022; .002)	.006	-.056; .106	-.010 (-.022; .002)	.006	-.055; .114
Physical activity	**-.400 (-.761; -.039)**	**.184**	**-.066; .030**	**-.402 (-.763; -.041)**	**.184**	**-.066; .029**
Lipids imbalance	**.739 (.203; 1.275)**	**.273**	**.081; .007**	**.704 (.153; 1.255)**	**.281**	**.078; .012**
Lipids imbalance X Age	–	–	–	-.017 (-.077; .044)	.031	-.017; .592
$F_{(df1;df2)}$; R^2; $R^2_{adjusted}$; *p*	$23.271_{(8; 934)}$; .166; .159; <.001			$20.701_{(9; 933)}$; .166; .158; <.001		
Age	.006 (-.037; .050)	.022	.009; .775	.004 (-.039; .047)	.022	.006; .852
Gender [a]	**-4.04 (-5.076; -3.004)**	**.528**	**-.317; <.001**	**-4.059 (-5.095; -3.024)**	**.528**	**-.318; <.001**
Formal education [b]	**-2.147 (-3.231; -1.062)**	**.553**	**-.123; <.001**	**-2.038 (-3.129; -.947)**	**.556**	**-.117; <.001**
Former smoker [c]	-.155 (-1.267; .957)	.567	-.01; .784	-.194 (-1.306; .918)	.567	-.013; .732
Smoker [c]	.398 (-1.269; 2.065)	.849	.015; .639	.395 (-1.27; 2.061)	.849	.015; .642
Alcohol consumption	-.009 (-.022; .003)	.006	-.051; .149	-.009 (-.022; .003)	.006	-.050; .153
Physical activity	**-.407 (-.77; -.044)**	**.185**	**-.067; .028**	**-.413 (-.775; -.050)**	**.185**	**-.068; .026**
Blood pressure	-.100 (-.672; .472)	.291	-.011; .731	-.075 (-.647; .497)	.292	-.008; .797
Blood pressure X Age	–	–	–	-.053 (-.115; .010)	.032	-.050; .098
$F_{(df1;df2)}$; R^2; $R^2_{adjusted}$; *p*	$22.201_{(8; 934)}$; .160; .153; <.001			$20.075_{(9; 933)}$; .162; .154; <.001		

[a] Gender, reference category: female. [b] Formal education measured in years, reference category: 4 years or less. [c] Smoking status, reference category: nonsmoker. [d] Alcohol consumption measured in gr/day, [e] Physical activity in number of times per week, reference category: none.

Supplementary table 3 - Linear regression the quadratic term of obesity in mood and moderation analysis of age.

GDS score						
	B (CI 95%)	**SE**	**β; *p***	**B (CI 95%)**	**SE**	**β; *p***
Age	.002 (-.041; .045)	.022	.003; .935	.000 (-.043; .043)	.022	.000; .993
Gender [a]	**-3.922 (-4.961; -2.883)**	**.529**	**-.307; <.001**	**-3.992 (-5.029; -2.955)**	**.528**	**-.313; <.001**
Formal education [b]	**-2.063 (-3.145; -.981)**	**.551**	**-.119; <.011**	**-2.063 (-3.142; -.984)**	**.550**	**-.119; <.001**
Former smoker [c]	-.169 (-1.281; .943)	.567	-.011; .766	-.145 (-1.254; .964)	.565	-.010; .798
Smoker [c]	.321 (-1.342; 1.983)	.847	.012; .705	.509 (-1.153; 2.172)	.847	.020; .548
Alcohol consumption	-.010 (-.022; .002)	.006	-.055; .110	-.009 (-.021; .003)	.006	-.051; .142
Physical activity	**-.380 (-.742; -.018)**	**.184**	**-.063; .040**	**-.419 (-.781; -.057)**	**.184**	**-.069; .023**
Obesity	.134 (-.339; .607)	.241	.018; .578	.118 (-.356; .592)	.242	.016; .626
Obesity^2	**.434 (.099; .769)**	**.171**	**.081; .011**	**.468 (.115; .82)**	**.180**	**.087; .009**
Obesity X Age	–	–	–	**-.077 (-.131; -.023)**	**.027**	**-.090; .005**
Obesity^2 X Age	–	–	–	.021 (-.021; .064)	.022	.033; .331
$F_{(df1;df2)}$; R^2; $R^2_{adjusted}$; *p*	$22.884_{(8;\ 934)}$; .164; .157; <.001			$17.872_{(11;\ 931)}$; .174; .165; <.001		

CHAPTER IV

Neuro-cognitive temporal dynamics and metabolic biomarkers following bariatric surgery.

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(In preparation)

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ABSTRACT

Background: Bariatric surgery has been shown to be the best method for achieving sustained weight loss in severe obesity. In addition, there is increasing evidence that weight loss following bariatric surgery is accompanied by sustained improvement in anxiety, mood, memory and executive function. It is recognized that metabolic surgery induces large metabolic changes, so we elected to study the longitudinal dynamics of the associations between metabolic markers, mood and cognition following bariatric surgery.

Methods: 110 participants were enrolled in the study. One to three assessments were performed for each participant, separated by at least 12 months and included anthropometrics, mood and cognitive performance. During the assessments blood was collected to analyze relevant metabolic biomarkers.

Results: HADS-anxiety and HADS-depression scores evolved similarly with time, reaching the lowest levels shortly after the surgery but then increasing over time to levels similar to those observed before the surgery. As predicted, several metabolic markers were significantly reduced when compared with pre-surgery levels, and some were correlated with changes in anxiety, mood, memory and cognition. It was found that both the trends and strength of the associations were a function of time elapsed since surgery.

Conclusion: Metabolic biomarkers are able to be correlated with anxiety, depressive mood and cognitive performance in bariatric surgery patients. Associations were found not to be stable but rather were seen to evolve as the interval following surgery increased.

Keywords: Bariatric Surgery, Anxiety, Mood, Cognition, Metabolism

INTRODUCTION

Obesity is increasing world-wide at an alarming rate, especially in developed countries [1]. Besides the already well-known complications of obesity, such as type 2 diabetes mellitus, hypertension, cardiovascular disease and others [2], obesity is also associated with debilitating neuro-psychosocial consequences such as depression and anxiety [3]. Clinical interviews of candidates for bariatric surgery suggest that 20 to 70% suffer from or had suffered from some form of psychiatric disability, depression and anxiety the most frequently mentioned [4]. Furthermore, obesity has also been associated with poorer cognitive performance in bariatric surgery candidates [5,6].

Currently, bariatric surgery is the best method of achieving substantial sustained weight loss in severely obese [7,8]. Most of the comorbidities of obesity show a reversal or amelioration after bariatric surgery. Several studies offer evidence that improvement of metabolic abnormalities, such as type 2 diabetes mellitus, can be achieved as a consequence of bariatric surgery [9]. Furthermore, it has been demonstrated that metabolic surgery is associated with an improvement in psychological status [10]. These findings are particularly interesting in the case of anxiety and depression. For those conditions improvement might be termed dose-dependent, meaning that larger excessive weight loss is associated with greater improvement in symptoms [10]. Deficits in memory and executive function may contribute to poorer adherence to post surgery guidelines and impair the results of operation [5]. In addition, there is increasing evidence that weight loss following bariatric surgery is associated with sustained improvements in memory, executive function and cognitive control [6].

The mechanisms of action by which bariatric surgery works are still an enigma. The explanation of created malabsorption (via by-pass) and restriction (via sleeve gastrectomy) have been shown not to be sufficient to explain the results of bariatric surgery [8]. More recently, hormonal changes have been implicated in the explanation of the results of bariatric surgery [8,11]. The most well studied hormones in the relevant studies are leptin, ghrelin and insulin. While several of these hormones, such as leptin and insulin, have been shown to fall after all forms of bariatric surgery [8,12], others, such as ghrelin, have been less predictable [8,13]. Beyond their effects on appetite regulation and metabolism, these hormones have been studied as potential mediators of the observed association between adiposity and psychological [14] or cognitive function [15,16].

Bariatric surgery induces significant alterations in body composition and concomitant metabolic alterations which might affect anxiety, mood and cognition. Furthermore, metabolic biomarkers, anxiety,

mood and cognition all are recognized to vary with time, which may indicate that the associations between these factors are dynamic. No study thus far published has addressed possible temporal dynamics in the association between hormonal milieu with mood and cognition following bariatric surgery. For this reason, we designed and carried out the present longitudinal investigation.

SUBJECTS AND METHODS

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by local ethics review boards. The goals and nature of the tests were explained to potential participants and all volunteers provided informed written consent.

Study sample

Subjects

Participants were recruited during their evaluation (surgery, psychiatry and dietetics/nutrition) for bariatric surgery or in post-surgery evaluations. Those ultimately enrolled in the present study (n=110) were recruited from Hospital de Braga and were either candidates for (n=30) or patients (n=77) who had already undergone bariatric surgery (either Sleeve gastrectomy or Roux-en-Y bypass). Exclusion criteria at the first assessment included inability to understand informed consent, incapacity and/or inability to attend the evaluation/assessment session(s), diagnosed neuropsychiatric disorder, age under 18 or greater than 65, had previously performed bariatric surgery more than 60 months previously and/or more than one bariatric intervention. From the enrolled participants 3 were excluded (gastric banding, n=2; illiterate, n=1) (Figure 1). Pre-surgery participants were reassessed at varying points in time after surgery, the post-surgery participants were re-assessed with a mean interval of one year or when available for the assessment. One to three assessments were performed for participant.

A demographic and clinical evaluation was performed during each assessment. Gender, age and education (years of formal education) were used as control variables.

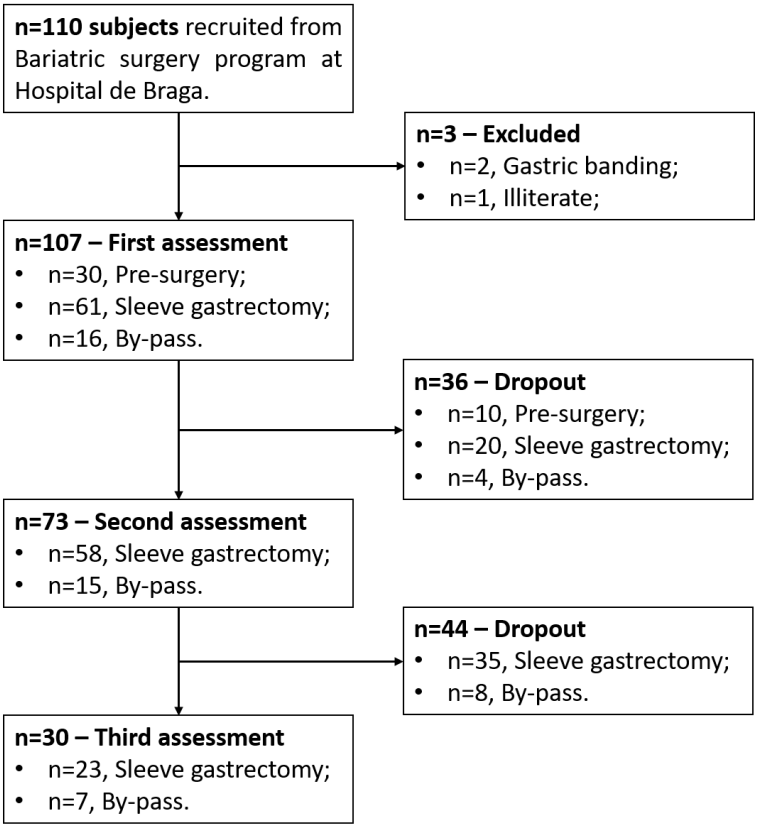

Figure 1 – Flow diagram of participant's recruitment and assessments.

Anthropometric assessment

Height and weight were measured before assessment for all participants and used to calculate the body mass index (BMI; BMI (kg/m^2) = weight (kg) / height $(m)^2$). Pre-surgery weight of the patients that had already undergone operation was obtained from medical records. Percentage of excessive weight loss (%EWL) was calculated as the relative amount of weight lost that was needed to achieve a BMI of 25 kg/m^2.

Neurocognitive assessment

Depressive mood, anxiety symptomatology memory and executive function were assessed using validated instruments by an experienced psychologist. Depressive mood and symptoms of anxiety during the previous week were assessed using Hospital Anxiety and Depression Scale (HADS) [17,18]. In order to assess cognitive function, multiple trial verbal learning and memory evaluation was performed with the Selective Remanding Test [19] [SRT, parameters: consistent long term retrieval (CLTR), long term storage (LTS),

delayed recall (DR)], list A and B were applied alternately to minimize the learning effect, and for the assessment of response inhibition/cognitive flexibility we used the STROOP color and word test [20] (parameters: Words, Colors and Colors/Words). Stroop interference was calculated as: Stroop interference = CW − [(W × C)/(W + C)] where CW: number of items properly named in 45s in the Colors/Words condition; W: number of items properly named in 45s in the Words condition; C: number of items properly named in 45s in the Colors condition [21].

Metabolic biomarkers assessment

Immediately before the psychological and cognitive assessment blood was collected by venipuncture, then centrifuged and serum separated. Serum samples were immediately frozen and stored at -80 °C in order that all samples would be analyzed under the same conditions.

The levels of 10 biomarkers; C-peptide, ghrelin, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon, insulin (circulating insulin), leptin, total plasminogen activator inhibitor-1 (PAI-1), resistin and visfatin, were analyzed using the Bio-Plex Pro Human Diabetes 10-Plex Panel (Bio-Rad Laboratories, Hercules, CA, USA). Serum quantification was performed according to the manufacturer's protocol using the BioPlex®200 Multiplex System platform (Bio-Rad Laboratories) and the data were automatically analyzed and processed using Bio-Plex Manager 6.1 software (Bio-Rad Laboratories).

Statistical analysis

To evaluate normal distribution of the variables, skewness and kurtosis values were calculated and the approximate normal distribution was defined for variables with absolute values of skewness below 3 and of kurtosis below 8. Log transformations were performed to normalize the distribution of skewed distributed variables before statistical tests were performed. Participants' characteristics regarding normally distributed variables are presented in mean and standard deviation (M; SD) in Tables, and/or mean and standard error of mean in Figures. Data from not normally distributed variables is presented in median and interquartile range (Tables and Figures).

A Memory component composed of the SRT (CLTR, LTS and DR) parameters was obtained using principal component analysis to reduce the number of variables with a minimum loss of information. The reliability

of the component was analyzed using Cronbach's alpha (Cronbach's alpha: 0.811). Component scores were obtained (using the regression method) and then used in subsequent analysis.

To assess the evolution of interest variables with time, each evaluation session was assigned to the one of the following time intervals: pre-surgery, 1 to 6 months (after surgery), 7 to 18 months, 19 to 36 months and >36 months. Linear mixed models were used to test the differences between time categories in order to account for intra individual correlation. Pre-surgery was considered the reference time point and the differences with subsequent epochs were tested with *Bonferroni* correction and statistical significance was defined at $p < .05$ level.

The presence of potential changes in anxiety, mood and cognition was investigated by applying generalized estimating equations (GEE) with repeated measures. This statistical method considers the multiple observations per subject which are likely to be correlated and treats them as clusters. The dependent variables were HADS-anxiety, HADS-depression, memory and STROOP interference. The independent variables were time (included as a continuous variable), metabolic biomarkers levels, and the interaction of metabolic biomarkers with time. Gender (female as reference), age, education, BMI and surgery (pre-surgery as reference; versus Sleeve gastrectomy or Roux-en-Y bypass) were included as covariates in the analyses. The GEE analysis yields coefficients which represent the associations between the dependent variable and the independent variables included in the model. We identified three primary coefficients of interest for each model: one relating the change in dependent variables with time, one relating the change in dependent variables with metabolic biomarkers, and an interaction term for time and metabolic biomarkers. A significant *p* value for the time coefficient reflects a statistically significant change in dependent variables over the total duration of follow-up. A significant *p* value for the coefficient for the metabolic biomarkers indicates a significant association of the dependent variables with metabolic hormone levels at the beginning of the study. A significant p value for the interaction coefficient represents a significant change in the association of the dependent variable with a metabolic biomarker over time. For the propose of these analyses we focused on the statistical significance of the metabolic biomarker's coefficient and on the interaction coefficient at a statistical significance defined at the $p < .01$ level. Statistical analysis was conducted using the IBM SPSS Statistics v25.

RESULTS

Sample characterization

As shown in Figure 1, at the first the inclusion, 30 participants were bariatric surgery candidates, 61 had been submitted to a sleeve gastrectomy and 16 to a Roux-en-Y bypass. From the 107 participants considered for the first assessment, 92 (86%) were females. The mean age at inclusion was 42 years (M=42.065; SD=11.264) and mean education level was 10 years of formal education (M=9.55; SD=3.747). Mean age of inclusion for the pre-surgery group was 39 years (M=39.2; SD=11.657), 42 years for the sleeve gastrectomy group (M=41.656; SD=10.915) and 49 years for the Roux-en-Y bypass (M=49.000; SD= 11.265). Pre-surgery group presented a higher educational level (M=10.3; SD=3.564) followed by the sleeve gastrectomy group (M=9.85; SD=3.750) and Roux-en-Y bypass (M=7.0; SD=3.162). The mean time of inclusion following surgery was 15 months (M=15.336; SD=14.737) for the participants that had undergone sleeve gastrectomy and 12 months (M=11.625; SD=11.348) for those who had undergone Roux-en-Y by-pass. At our first assessment sleeve gastrectomy participants had lost a mean of 68% (M=68,398; SD=20.370) of the excessive weight and the participants from the Roux-en-Y bypass had lost a mean of 61% (M=60,909; SD=23.947) of the excessive weight.

Prospective changes in neurocognitive performance and metabolic biomarkers

During the course of the study a total of 210 evaluation sessions were performed. In order to investigate how the assessed parameters changed with time, each evaluation session was placed in one of the following time bins: pre-surgery, 1 to 6 months (after surgery), 7 to 18 months, 19 to 36 months and >36 months. Table 1 shows the evolution of socio-demographic and anthropometric characteristics over the course of follow-up (note that each subject can contribute to up to 3 epochs of time). Comparison of pre-surgery BMI with the subsequent time categories reveals a significant decrease for all intervals ($p<$ .001).

Table 1 – Participants characterization by time categories.

	Pre-surgery (n=30)	1 to 6 months (n=37)	7 to 18 months (n=49)	19 to 36 months (n=51)	> 36 months (n=43)
Socio-demographic					
Gender (n; %)					
Female	25; 83.33	31; 83.78	40; 81.63	44; 86.27	39; 90.69
Male	5; 16.67	6; 16.22	9; 18.37	7; 13.73	4; 9.31
Age (M; SD) (years)	39.2; 11.657	42.649; 11.051	40.163; 11.172	46.706; 10.116	46.814; 11.45
Education (M; SD) (years)	10.3; 3.564	9.459; 3.54	9.612; 3.481	9.294; 3.679	8.953; 3.86
BMI (M; SD) (kg/m2)	43.906; 8.28	34.647; 5.294	30.206; 5.276	29.99; 3.538	31.839; 3.618
%EWL (M; SD) (%)	–	52.265; 18.217	77.178; 19.409	75.139; 15.238	67.113; 15.23

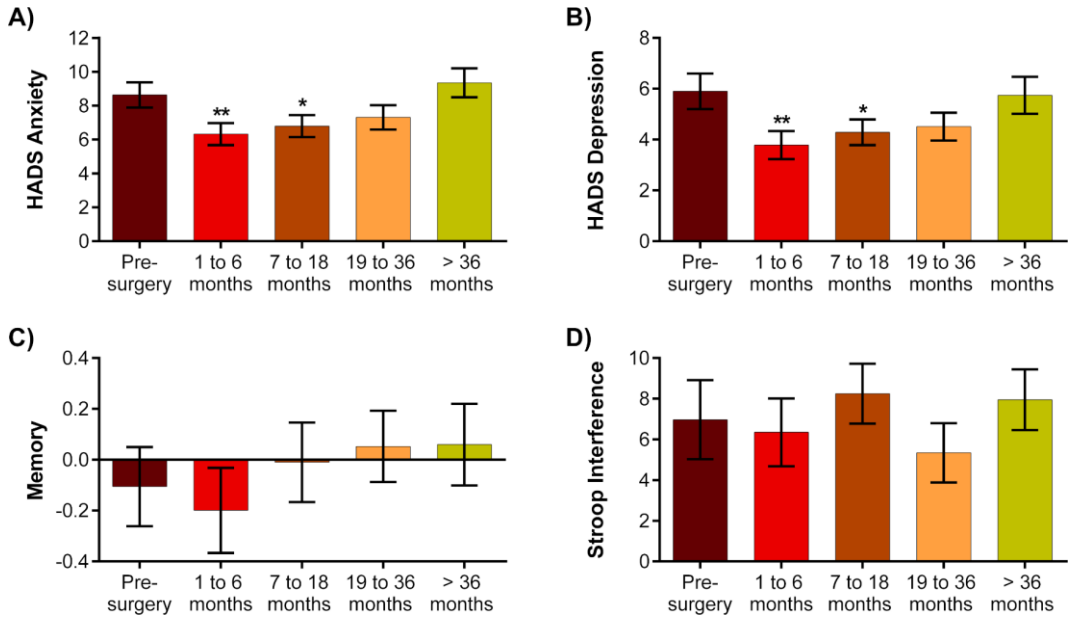

Figure 2 – Neuro-cognitive performance across time categories. A) HADS-anxiety score. **B)** HADS-depression score. **C)** Memory factor score. **D)** Stroop interference score. Data presents in mean and standard error of mean. * p<.05; ** p<.01; after Bonferroni multiple comparisons correction.

Figure 2 and Supplementary Table 1 show the evolution of neurocognitive variables over time. HADS-anxiety and HADS-depression scores showed a similar pattern, reaching the lowest levels shortly after the surgery and increasing over time to levels similar to those observed before the surgery (Figure 2A and 2B). A significant reduction in HADS-anxiety and HADS-depression scores from pre-surgery to the 1 to 6 months post-operation was observed (p< .01). Those differences decreased, but remained significant, in the group 7 to 18 months following surgery (p< .05). Anxiety and depression scores in the interval 19 to

36 months and > 36 months after operation were not significantly different from those in the pre-surgery group. No significant differences as a function of time were observed for the memory score (Figure 2C) or STROOP interference score (Figure 2D).

Figure 3 and Supplementary Table 1illustrate the longitudinal progression of metabolic biomarkers over the time. C-peptide, insulin, ghrelin, glucagon, leptin and total PAI-1 all showed a significant reduction for all time periods when compared with pre-surgery levels (Figure 3A, 3B, 3C, 3F, 3G and 3H).No significant differences from pre-surgery levels were observed for GIP and GLP-1 levels (pre-surgery *vs* any other time category – $p > .05$) (Figure 3D and 3E). Shortly after surgery (1 to 6 months post-operation), resistin levels were not significantly different from the pre-surgery levels ($p > .05$), but a significant reduction was observed for later time intervals (pre-surgery *vs* 7 to 18 months – $p < .05$; pre-surgery *vs* 19 to 36 months or > 36 months – $p < .01$) (Figure 3I). Visfatin levels were diminished only the after 36 months when compared to the pre-surgery levels (pre-surgery *vs* >36 months – $p < .05$) (Figure 3J).

Longitudinal association of metabolic biomarkers and neurocognitive performance

GEEs were used to assess the association of time and metabolic biomarkers with the neurocognitive variables, and to address the impact of time upon participants performance. All the analysis were controlled for age, gender, education, BMI and surgical status.

Figure 4 and Supplementary Table 2 present the results of the association of metabolic biomarkers with neurocognitive variables. Before surgery, despite a negative tendency, no statistically significant association of C-peptide levels with HADS-anxiety was observed. Nevertheless, time had a significant impact on the strength and direction of this association, which became more positive with time. Similarly, before surgery glucagon was not significantly associated with HADS-anxiety, although, the association became stronger and positive with the passage of time (Figure 4A).

HADS-depression (Figure 4B) was negatively associated with insulin and GIP before surgery and time did have a significant impact on the strength and direction of the associations. It would thus appear that the negative associations are mor prominent at the time of the surgery and become less negative, or even positive, with time.

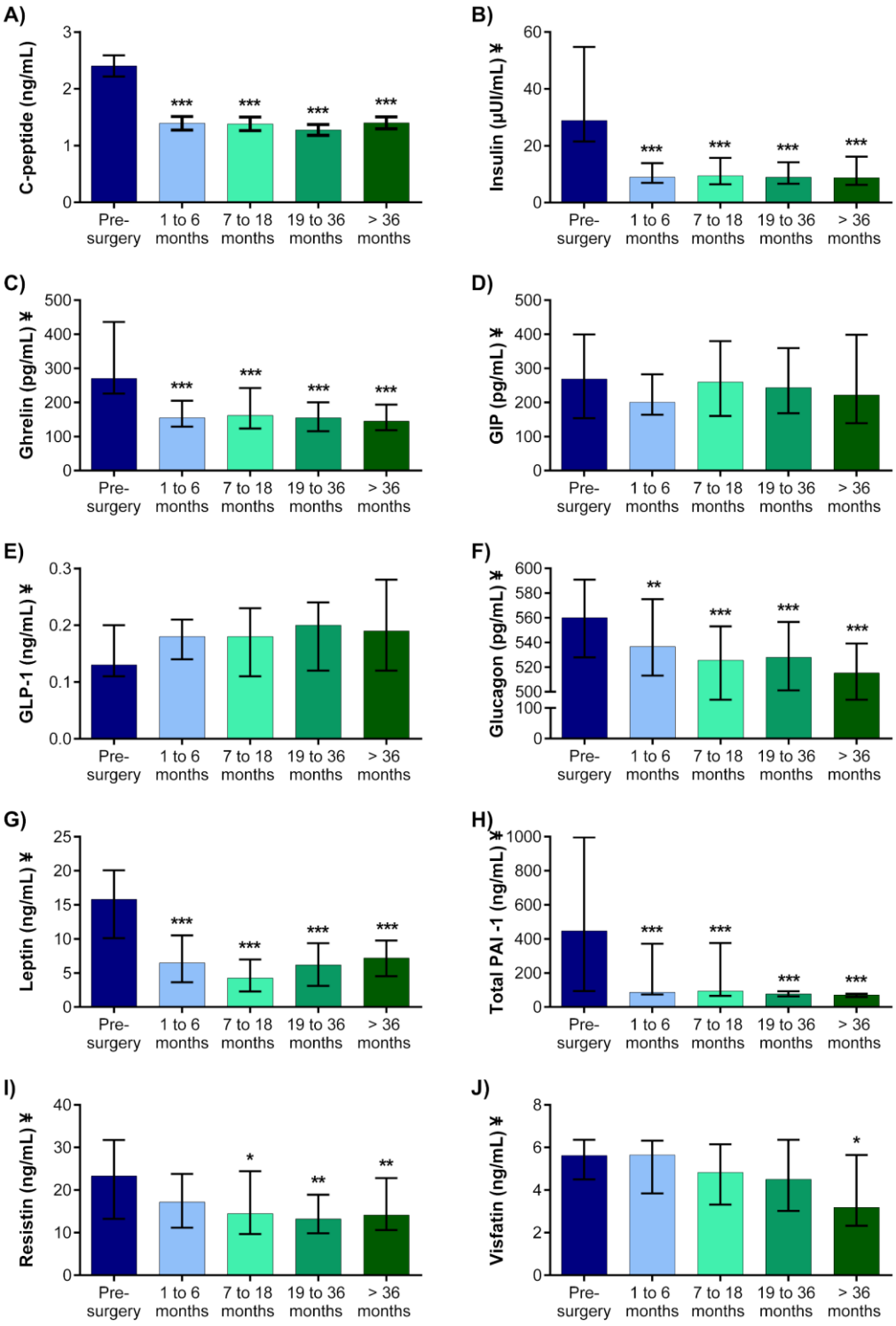

Figure 3 – Metabolic biomarkers levels across time categories. A) C-peptide levels (ng/mL). **B)** Insulin levels (µUI/mL). **C)** Ghrelin levels (pg/mL). **D)** GIP levels (pg/mL). **E)** GLP-1 levels (ng/mL). **F)** Glucagon levels (pg/mL). **G)** Leptin levels (ng/mL). **H)** Total PAI-1 levels (ng/mL). **I)** Resistin levels (ng/mL). **J)** Visfatin levels (ng/mL). Data presents in mean and standard error of mean or (¥) in median and interquartile range. * p<.05; ** p<.01; *** p<.001; after Bonferroni multiple comparisons correction.

A negative and significant association between HADS-depression and glucagon level was observed and at the onset was not significantly moderated by time remaining negative over the entire follow up period. In contrast before surgery, no association between HADS-depression and resistin was evident; yet with time the impact became significant and positive.

Before surgery the association between insulin level and memory function (Figure 4C) failed to reach significance (although a negative tendency was observed). GIP was found to be negatively associated with memory prior to operative intervention. For both those metabolic biomarkers, however memory function was significantly moderated by the passage of time, becoming less negative and eventually positive in longer follow-up intervals.

Before surgery, neither GIP (despite a positive tendency) nor glucagon were associated with STROOP interference, yet a significant negative interaction was observed with increasing time, reflecting an increasing effect (Figure 4D).

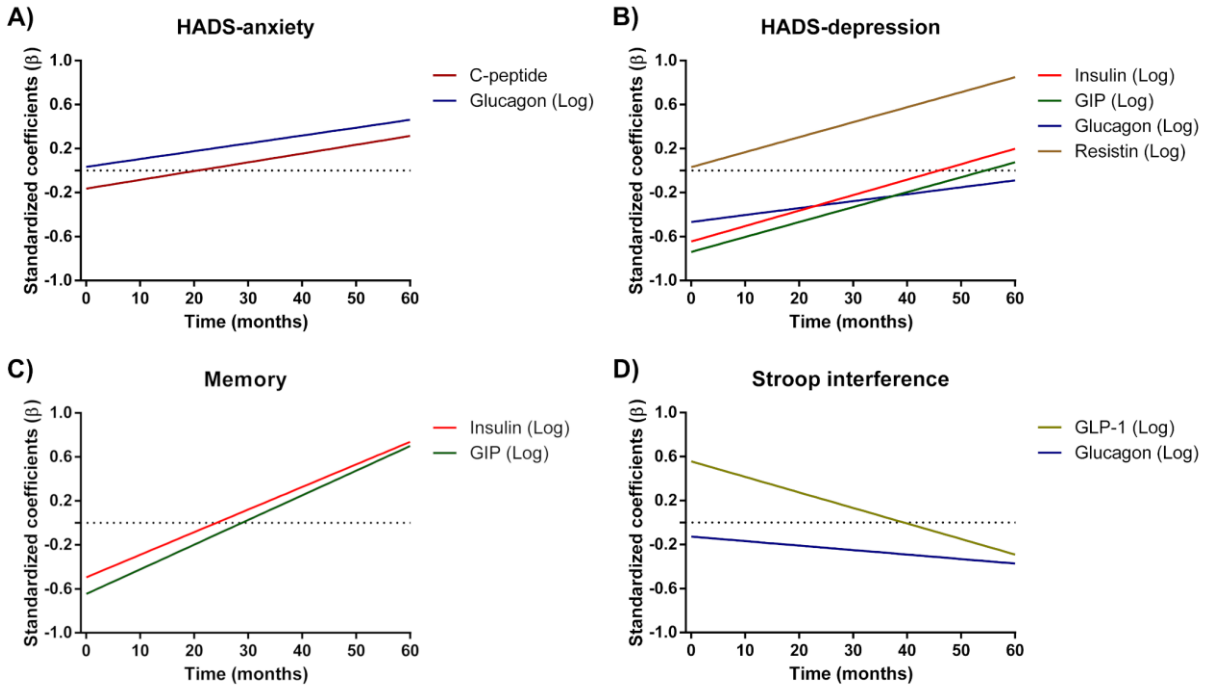

Figure 4 – Temporal dynamics of the association between neuro-cognitive performance and metabolic biomarkers. A) Temporal dynamics of the association between HADS-anxiety score, C-peptide and glucagon levels. **B)** Temporal dynamics of the association between HADS-depression score, insulin, GIP, glucagon and resistin levels. **C)** Temporal dynamics of the association between memory factor score insulin and GIP levels. **D)** Temporal dynamics of the association between Stroop interference score, GLP-1 and glucagon levels.

DISCUSSION

In this work we explore for the first time the temporal dynamics of the longitudinal association of a number of metabolic biomarkers with anxiety, depressive mood and cognition (memory and executive function). Our results support the hypothesis that the association of several metabolic biomarkers with neurocognitive performance is dynamic and changes with time after the intervention of bariatric surgery.

Previous reports [22,23] amply demonstrate that bariatric surgery results in a significant weight loss. In systematic reviews, Chang *et al.* [22] indicated that BMI loss at 5 years ranged from 12 to 17 kg/m^2 and Puzziferri *et al.* [23] reported that in long term follow-up studies the amount of excessive weight loss exceeds 50%. Similarly, our results show a BMI reduction of 12 to 14 kg/m^2 after surgery and excessive weight loss ranging from 52 to 77% during the follow-up.

Changes in metabolic biomarker levels as a result of bariatric surgery also are widely reported. As others have reported, we observe a postoperative decrease in ghrelin [8,24,25], glucagon [25], leptin [8,24], total PAI-1 [26,27] and resistin [24]. In addition, an improvement in glucose homeostasis was reflected in the decrease of c-peptide [28] and insulin [8,25,29,30] levels. Inconsistent results for the effect of bariatric surgery on GIP [11,31,32] and GLP-1 [11,25] levels has been reported. In our investigation, postoperative levels of GIP and GLP-1 were not significantly different from those of pre-surgery. Visfatin has been infrequently studied in bariatric surgery and those results reported have been inconsistent [33,34]. We found a significant decrease in levels only after 36 months when compared with those determined pre-operatively.

Anxiety and depressive mood indicators decreased shortly after the surgery and remained at significantly lower levels for up to 18 months. After this period, both anxiety and depressive mood did not differ from pre-surgery levels. Previous studies have described a maintenance [35-37] or decrease [38-40] in anxiety levels after surgery. Consistent with our results, Burgmer *et. al.* [38] reported that anxiety scores were significantly lower approximately 14 months after surgery when compared with pre-surgery levels and no significant differences were observed after this timepoint. Several lines of evidence have been advanced to explain improvement in depressive mood after bariatric surgery [35-37,41,42]. Most of the studies that have reported a decrease in depressive mood have had relatively short follow-up times (12 to 36 months) [35-37,42]. Burgmer *et. al.* [38] reported that approximately 5 years after surgery, depressive symptoms remained at a significantly lower level than those seen prior to surgery. On the other hand, Booth *et al.* [41], in a study whose follow-up interval was longer, reported a reduction in clinical depression and antidepressant use in the 2 first years after surgery, but not after the fourth year, after which there was no longer evidence

for significant improvement. Unfulfilled expectations that life will dramatically change as a consequence of surgery can negatively impact psychological health and potentially explain the longitudinal trajectory of anxiety and depressive mood here reported. Despite the initial improvement, bariatric patients may well be vulnerable to not having reached their aspirations and thus profit from ongoing attention to their mental health issues in order to maximize emotional gains after surgery [35,43].

Several works have previously reported that cognitive function improves with weight loss after bariatric surgery and that the observed improvements are maintained over time [5,6,44-46]. In our analysis, no significant changes in memory or executive function were seen following surgery. Visual inspection of the graphical representation of memory score as function of time suggests a trend with direction of memory improvement, but it does not reach statistical significance. In our analysis, executive function, as measured by Stroop interference, also did not change significantly with time. Georgiadou *et al.* [47] found no performance differences in several cognitive tests, including the Stroop test and others which assess memory, between individuals who had undergone bariatric surgery and those of age and gender-matched candidates for surgery. Also, Smith et al. [48] found no short-time improvement in memory or composite cognitive score either in Roux-en-Y gastric bypass or vertical sleeve gastrectomy patients. In a meta-analysis, Handley *et al.* [6] point out that a number of the published works regarding changes in cognitive function after bariatric surgery result from a single multisite study, the Longitudinal Assessment of Bariatric Surgery (LABS). This potential over-representation of one patient sample from which a large amount of data and multiple publications have been produced has potential to introduce certain level of bias to the literature, yet it also is important to recognize that studies of different populations have found similar results. In the present study, we are aware that the performance of participants in the cognitive tasks during every evaluation is high and that the general education level also is high, both having the potential to impact tested cognitive performance. On this basis, we recognize that the absence of improvements in cognition may be the result of a "ceiling effect". We also cannot rule out selection bias on the basis a greater willingness of those participants with higher cognitive performance agreeing to participate in the study and adhere to the follow-up schedule.

Several lines of evidence point to an association of the metabolic biomarkers here explored with anxiety [49,50], mood [49-58] and cognitive performance [44,56,59-61]. Several studies addressing these relationships have been performed in nom-surgical populations. Those which were done in bariatric patients did not address the fact that during the time elapsed following surgery, massive changes in the metabolic and hormonal

environment occur which may influence strength or the trend of the above-mentioned associations. In our investigation, we found that several metabolic biomarkers are associated with anxiety, mood and cognition, that the relationship is dynamic and changes with time.

To the best of our knowledge, no studies have explored the association of anxiety and C-peptide or glucagon in patients undergoing bariatric surgery. High levels of both are observed in insulin resistance and type II diabetes [62]. In turn, each of these conditions has been associated with anxiety [63]. Bariatric surgery has consistently been shown to lead to improvement in glucose homeostasis [29,64]. Despite of the absence of a statistically significant association, in our study population, between HADS-anxiety and C-peptide or glucagon before surgery, over time higher levels of these two metabolic biomarkers tended to be associated with higher scores in anxiety symptomatology and *vice versa*. The findings lead us to the observation that, at a time when glucose homeostasis should be improved, levels of C-peptide and glucagon which are suggestive of impaired glucose homeostasis are associated with higher anxiety levels.

Insulin, GIP and glucagon were negatively associated with depressive mood before surgery. While the relation of insulin and GIP to depression tended to become less negative or null across time, the association of glucagon remained negative with the passage of time. It is important to point out that metabolic biomarker levels were determined before the administration of the behavioral and cognitive tests and not under fasting conditions. As consequence, these levels of insulin do not reflect insulin resistance. While insulin resistance has been associated with depressive mood [56], intranasal insulin administration has improved mood even in insulin resistant subjects [65,66]. GIP can cross the blood-brain-barrier and data from animal models indicates that GIP plays a neuroprotective role by protecting synapse function and numbers, promoting neuronal proliferation and reducing the chronic inflammation response of the central nervous system [67]. These observations would be consistent the negative association here observed between GIP and depressive symptomatology. Glucagon has also been shown to be neuroprotective [68,69]. Consistent with our findings, lower levels of glucagon have been observed in the cerebrospinal fluid of those attempting suicide compared to healthy controls, while in the suicide attempting group the severity of depression was negatively associated with plasma glucagon levels [70]. Resistin was not associated with depressive mood before bariatric surgery but a positively associated over time. In line with our findings, Weber-Hamann *et al.* [71] found a decrease in resistin levels in treatment-responsive depressed patients, while no differences where observed for non-remitters. Similarly, Lehto *et al.* [72] found that resistin levels were correlated with atypical depressive symptoms but not with typical

depressive symptoms. While role of resistin in depression and depressive symptomatology is incompletely understood, *in vitro* studies demonstrated an ability to inhibit dopamine and noradrenaline release in the hypothalamus, which could potentially provide a link to depressive symptomatology [73].

Prior to surgery and before the subsequent metabolic improvement we observed a negative trend and association between memory function and insulin and GIP, respectively. Beginning at 30 months after operation surgery and increasing with longer follow-up, the association became positive, with higher levels of insulin and GIP associated with improved memory performance. Insulin resistance has been associated with impaired memory [56,74], and the central effects of insulin have been reported to improve memory [56]. Bariatric surgery has been consistently associated with improvements in insulin sensitivity and even with the remission of insulin resistance [64]. We therefore postulate that we may be observing an effect of the metabolic transition. Galioto *et al.* [75] have shown that, over time, while HOMA-IR decreased, working memory improved. The neuroprotective effects of GIP also have been observed in studies of memory [67], which is in accordance with the positive association we observed in the later periods after bariatric surgery. A loss of the incretin effect is observed in patients with type two diabetes, possibly due to a downregulation of the islet GIP receptor [76,77]. Data from GIP receptor knock-out animal models have demonstrated impaired memory and learning, as well as decreased synaptic plasticity and neurogenesis [78,79]. Taken together, these findings, led us to question whether the negative association here observed might not be the result of downregulation of the brain GIP receptor which is a possible consequence of certain peripheral metabolic complications.

Before surgery, GLP-1 trended to be positively associated with Stroop interference but the relationship vanished with time. Evidence from both pre-clinical studies and clinical trials point to a positive effect of GLP-1 on cognitive performance [80]. Research regarding the impact of glucagon on cognition is scarce and the sparse available literature suggests a neuroprotective effect [68,69]. Our results indicate a negative association between glucagon and the Stroop interference test at higher follow-up times. To the best of our knowledge, no studies have investigated the relationship between glucagon and executive function. Based on the available literature, the result here presented is unexpected and certainly requires confirmation. A possible explanation for the observed result may be related to the increased glucagon levels and delayed glucagon suppression seen in insulin resistance [62], and which as been associated with poorer executive function and diminished general cognitive performance [74].

The longitudinal nature of this work is one of its major strengths. The extensive characterization of neurocognitive performance and assays of numerous metabolic biomarkers, as well as analysis of the multiple factors which may affect the associations may also contribute to the strength of our findings. Much of the literature assessing the association of metabolic parameters with anxiety, mood and cognition uses fasting levels of the metabolic biomarkers. We chose to explore potential relationships by measuring the biomarker levels at the time of the neuro-cognitive evaluations. We recognize that the sample size, the inclusion of two types of bariatric surgery, different enrolment times in the study, and the number of repeated assessments may limit the interpretation of the results here described. Despite such limitations, our results and conclusions are generally consistent with previously published work, and serve to bolster published findings while advancing a number of new interpretations.

We have shown that anxiety and depressive symptomatology decrease as result of bariatric surgery, but return to the pre-surgery levels after 18 months. No relevant time-dependent alterations in cognitive performance were observed in our work. We suggest that despite initial improvement, bariatric patients well benefit from ongoing attention to mental health to maximize mental health gains after surgery. Bariatric surgery induces large metabolic alterations that, theoretically, can influence mood and cognition. We explored the temporal dynamics of the complex relationship between hormonal milieu, mood and cognition, which occurs following bariatric surgery. In addition, we demonstrated that several metabolic biomarkers are associated with anxiety, mood and cognition, and that the trend and strength of the association varies with time. A better understanding of the temporal dynamics of the association between metabolic biomarkers and anxiety, mood and cognition should make the design of personalized strategies to maximize mental health gains after bariatric surgery.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Contributions

CPN and JMB performed the statistical analysis. CPN, PC and JMB contributed to the data analyses and discussion. CPN and AF recruited the participants. CPN, LA and TCC collected the data. CPN wrote the first draft of the manuscript. NCS and conceived and designed the study. All the authors revised the manuscript. JMB had access to all the data in the study.

Acknowledgments

This work was funded by the Portuguese Foundation for Science and Technology (FCT) under the project PTDC/DTP-PIC/6936/2014. C.P.N. was supported by the Portuguese Science Foundation (FCT) doctoral scholarship PD/BD/106050/2015 via Inter-University PhD Programme in Ageing and Chronic Diseases. L.A. was a recipient of a doctoral fellowship from the Portuguese Foundation for Science and Technology (FCT; SFRH/BD/101398/2014). T.C.C. was a recipient of a doctoral fellowship from the Portuguese Foundation for Science and Technology (FCT; SFRH/BD/90078/2012). N.C.S. was a recipient of a Research Assistantship by the through the FCT Investigator Programme 200∞Ciência.

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SUPPLEMENTARY MATERIAL

Supplementary Table 3 – Neuro-cognitive and metabolic characterization by time categories.

	Pre-surgery (n=30)	1 to 6 months (n=37)	7 to 18 months (n=49)	19 to 36 months (n=51)	> 36 months (n=43)
Mood (M; SD)					
HADS-anxiety	8.633; 4.089	6.324; 3.916	6.796; 4.555	7.314; 5.124	9.349; 5.606
HADS-depression	5.9; 3.836	3.784; 3.368	4.286; 3.518	4.51; 3.911	5.744; 4.781
Cognition (M; SD)					
MEM Factor	-.106; .850	-.199; .989	-.01; 1.083	.052; 1.003	.059; 1.014
DSST	60.367; 23.563	50.314; 19.675	61.327; 21.062	55.157; 19.488	51.048; 22.568
STROOP Interference	6.97; 10.657	6.347; 9.853	8.244; 10.316	5.344; 10.301	7.95; 9.576
Metabolism (M; SD)					
C-peptide (ng/mL)	2.403; .968	1.392; .706	1.382; .825	1.276; .682	1.4; .682
Ghrelin (pg/mL) ¥	270.36; 210.62	154.96; 76.68	161.62; 118.94	154.92; 84.88	144.72; 75.06
GIP (pg/mL) ¥	268.92; 245.74	200.52; 118.88	259.64; 219.92	243.44; 191.48	221.92; 259.24
GLP-1 (ng/mL) ¥	.127; .093	.183; .074	.182; .124	.202; .119	.192; .159
Glucagon (pg/mL) ¥	560.08; 62.88	536.56; 61.92	525.47; 54.18	527.92; 55.52	515.08; 42.4
Insulin (µUI/mL) ¥	28.885; 33.25	8.946; 6.916	9.412; 9.308	8.903; 7.531	8.731; 9.866
Leptin (ng/mL) ¥	15.809; 9.946	6.484; 6.88	4.255; 4.692	6.188; 6.248	7.195; 5.247
Total PAI -1 (ng/mL) ¥	446.839; 902.299	86.444; 297.571	93.486; 309.809	76.688; 29.583	70.898; 19.292
Resistin (ng/mL) ¥	23.305; 18.511	17.188; 12.645	14.46; 14.743	13.211; 9.072	14.137; 12.205
Visfatin (ng/mL) ¥	5.615; 1.854	5.653; 2.487	4.829; 2.834	4.504; 3.334	3.185; 3.325

¥ - Variable not normally distributed, data is presented in median; interquartile range.

Supplementary Table 4 – Generalized estimating equations for the association between metabolic biomarkers and neurocognitive performance over time**.**

	HADS-Anxiety			HADS-Depression		
	B (99% CI)	**SE**	***p***	**B (99% CI)**	**SE**	***p***
C-peptide						
Time	**-.018 (-.097; .06)**	**.030**	**.548**	-.056 (-.151; .039)	.037	.129
C-peptide	**-.952 (-2.129; .225)**	**.457**	**.037**	-2.224 (-4.596; .147)	.921	.016
Time*C-peptide	**.046 (0; .093)**	**.018**	**.010**	.066 (-.008; .140)	.029	.021
Insulin						
Time	-.037 (-.215; .140)	.069	.589	**-.158 (-.294; -.021)**	**.053**	**.003**
Insulin (Log)	-.471 (-4.25; 3.308)	1.467	.748	**-8.249 (-12.73; -3.773)**	**1.738**	**<.001**
Time*Insulin (Log)	.078 (-.094; .250)	.067	.243	**.180 (.038; .321)**	**.055**	**.001**
Ghrelin						
Time	-.107 (-.874; .661)	.298	.720	-.089 (-1.029; .850)	.365	.807
Ghrelin (Log)	-1.114 (-8.411; 6.183)	2.833	.694	-2.415 (-12.24; 7.407)	3.813	.527
Time*Ghrelin (Log)	.070 (-.286; .426)	.138	.614	.045 (-.399; .489)	.172	.795
GIP						
Time	-.149 (-.398; .100)	.097	.122	**-.436 (-.799; -.074)**	**.141**	**.002**
GIP (Log)	-1.761 (-4.959; 1.437)	1.242	.156	**-10.37 (-16.79; -3.951)**	**2.491**	**<.001**
Time*GIP (Log)	.079 (-.019; .177)	.038	.039	**.190 (.034; .347)**	**.061**	**.002**
GLP-1						
Time	.043 (-.074; .160)	.045	.343	.164 (-.039; .366)	.079	.037
GLP-1 (Log)	1.070 (-4.238; 6.379)	2.061	.603	-6.62 (-16.316; 3.077)	3.764	.079
Time*GLP1 (Log)	.010 (-.123; .143)	.052	.848	.204 (-.029; .438)	.091	.024
Glucagon						
Time	**-1.423 (-2.651; -.194)**	**.477**	**.003**	**-1.076 (-2.279; .127)**	**.467**	**.021**
Glucagon (Log)	**2.591 (-7.614; 12.80)**	**3.962**	**.513**	**-28.94 (-51.76; -6.108)**	**8.862**	**.001**
Time*Glucagon (Log)	**.541 (.091; .991)**	**.175**	**.002**	**.391 (-.055; .836)**	**.173**	**.024**
Leptin						
Time	.002 (-.072; .075)	.029	.954	-.008 (-.057; .041)	.019	.671
Leptin (Log)	1.627 (-1.121; 4.375)	1.067	.127	-1.862 (-7.007; 3.283)	1.997	.351
Time*Leptin (Log)	.067 (-.019; .153)	.033	.044	.044 (-.072; .159)	.045	.331
PAI-1						
Time	.035 (-.167; .237)	.078	.657	-.205 (-.535; .125)	.128	.110
PAI-1 (Log)	-.451 (-2.155; 1.253)	.662	.495	-.677 (-3.924; 2.569)	1.260	.591
Time*PAI-1 (Log)	.002 (-.105; .108)	.041	.970	.129 (-.063; .322)	.075	.082
Resistin						
Time	-.001 (-.176; .175)	.068	.993	**-.167 (-.300; -.034)**	**.052**	**.001**
Resistin (Log)	.090 (-3.294; 3.474)	1.314	.945	**.434 (-4.413; 5.28)**	**1.882**	**.818**
Time*Resistin (Log)	.044 (-.107; .195)	.059	.451	**.188 (.050; .325)**	**.053**	**<.001**
Visfatin						
Time	.190 (-.064; .445)	.099	.054	-.009 (-.126; .107)	.045	.835
Visfatin (Log)	11.63 (-5.526; 28.78)	6.659	.081	-1.321 (-1.257; 7.614)	3.469	.703
Time*Visfatin (Log)	-.130 (-.427; .167)	.115	.260	-.002 (-.138; .133)	.053	.964

All the analysis were controlled for gender (female as reference), age, education, BMI and surgery (pre-surgery as reference).

Supplementary Table 2 (continuation) – Generalized estimating equations for the association between metabolic biomarkers and neurocognitive performance over time**.**

	Memory			STROOP Interference		
	B (99% CI)	**SE**	***p***	**B (99% CI)**	**SE**	***p***
C-peptide						
Time	-.051 (-.102; -.001)	.020	.008	.124 (-.073; .32)	.0760	.105
C-peptide	-.369 (-1.012; .274)	.250	.140	1.521 (-2.59; 5.631)	1.5960	.341
Time*C-peptide	.013 (-.004; .030)	.007	.043	-.052 (-.167; .063)	.0450	.243
Insulin						
Time	**-.098 (-.140; -.057)**	**.016**	**<.001**	.147 (-.162; .455)	.120	.221
Insulin (Log)	**-1.59 (-3.463; .283)**	**.727**	**.029**	5.202 (-8.970; 19.38)	5.502	.344
Time*Insulin (Log)	**.066 (.024; .108)**	**.016**	**<.001**	-.101 (-.396; .193)	.114	.375
Ghrelin						
Time	.116 (-.184; .415)	.116	.320	-.814 (-2.934; 1.307)	.823	.323
Ghrelin (Log)	-.397 (-3.795; 3.001)	1.319	.763	5.438 (-9.074; 19.95)	5.634	.334
Time*Ghrelin (Log)	-.068 (-.206; .070)	.054	.205	.435 (-.585; 1.455)	.396	.272
GIP						
Time	**-.223 (-.346; -.100)**	**.048**	**<.001**	.745 (-.070; 1.560)	.316	.018
GIP (Log)	**-2.277 (-3.956; -.598)**	**.652**	**<.001**	11.84 (-2.167; 25.85)	5.438	.029
Time*GIP (Log)	**.079 (.030; .128)**	**.019**	**<.001**	-.303 (-.636; .031)	.130	.019
GLP-1						
Time	-.003 (-.071; .064)	.026	.903	**-.358 (-.773; .058)**	**.161**	**.027**
GLP-1 (Log)	-.871 (-3.175; 1.433)	.895	.330	**22.594 (-.894; 46.08)**	**9.119**	**.013**
Time*GLP1 (Log)	.044 (-.019; .108)	.025	.073	**-.573 (-1.011; -.135)**	**.170**	**.001**
Glucagon						
Time	-.493 (-2.379; 1.394)	.733	.501	**1.813 (.052; 3.575)**	**.684**	**.008**
Glucagon (Log)	-1.685 (-15.03; 11.66)	5.179	.745	**-2.113 (-6.042; 19.82)**	**15.50**	**.194**
Time*Glucagon (Log)	.170 (-.533; .873)	.273	.533	**-.645 (-1.276; -.014)**	**.245**	**.008**
Leptin						
Time	-.035 (-.080; .009)	.017	.040	-.003 (-.126; .120)	.048	.948
Leptin (Log)	-.163 (-1.558; 1.233)	.542	.764	-5.385 (-15.95; 5.176)	4.100	.189
Time*Leptin (Log)	.008 (-.047; .063)	.021	.712	.077 (-.111; .266)	.073	.291
PAI-1						
Time	-.042 (-.147; .062)	.040	.294	.706 (-.803; 2.216)	.5860	.228
PAI-1 (Log)	-.403 (-1.174; .368)	.299	.178	-.222 (-11.934; 11.49)	4.5470	.961
Time*PAI-1 (Log)	.006 (-.040; .051)	.018	.755	-.375 (-1.168; .417)	.3080	.223
Resistin						
Time	-.077 (-.138; -.016)	.024	.001	-.137 (-.584; .311)	.174	.431
Resistin (Log)	-.567 (-2.756; 1.621)	.850	.504	-6.363 (-24.78; 12.05)	7.148	.373
Time*Resistin (Log)	.042 (-.009; .092)	.020	.034	.129 (-.227; .484)	.138	.351
Visfatin						
Time	-.028 (-.075; .019)	.018	.124	.133 (-.186; .451)	.124	.284
Visfatin (Log)	-.031 (-2.816; 2.754)	1.081	.977	.936 (-14.23; 16.102	5.888	.874
Time*Visfatin (Log)	-.006 (-.058; .046)	.020	.758	-.020 (-.241; .200)	.086	.811

All the analysis were controlled for gender (female as reference), age, education, BMI and surgery (pre-surgery as reference):

CHAPTER V

Neural correlates of appetite information processing in major depression after 6 to 8 weeks of paroxetine treatment.

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(On going – preliminary results)

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ABSTRACT

Background: Appetite or weight disturbance is commonly observed in patients with major depressive disorder. Paroxetine is a selective serotonin reuptake inhibitor widely used antidepressants that impact on weight and appetite. Despite the largely recognized impact of depression and antidepressant medication on weight and appetite, studies exploring the impact of depression and antidepressants in appetite neural correlates are scarce. Here we explore the neural correlates of depression and antidepressant treatment with paroxetine in neural processing of appetite information.

Methods: 23 treatment naïve depressed patients and the treatment prescription was paroxetine 20 mg were recruited from the emergency room at Hospital de Braga and 16 healthy controls were recruited from the general population. Brain activity was measured using functional magnetic resonance during an appetite challenging task. Depression, anxiety, stress, eating behavior and anthropometric measures were collected. 19 patients were reassessed after 6 to 8 weeks of treatment with paroxetine.

Results: Compared to controls, depressed patients presented a decrease in brain activity in bilateral temporal regions and in right cerebellum. After treatment, patients presented increased brain activity in left lateral prefrontal areas, left anterior cingulate cortex and right insula. Furthermore, changes in the activity of those brain regions were associated with changes in body fat, perceived stress and eating behavior.

Conclusion: We observed that depressed patients present a decrease brain activation in areas associated with the processing of food stimuli (temporal cortex) and areas that integrate somatic responses to food (cerebellum). Areas associated with reward (lateral prefrontal cortex), decision-making (anterior cingulate cortex) and integration of sensory cues (insula) present a higher activation after 6 to 8 weeks of treatment with paroxetine. We also show that the difference in brain activity after treatment are associated with body composition, eating behavior and stress.

Keywords: Appetite, Treatment-Naïve, Depression, Paroxetine, Brain activity

INTRODUCTION

Depression is a highly prevalent and debilitating condition, making it an important global public-health issue [1]. In fact, depressive disorders are the third leading cause of non-fatal health loss and this has been true for at least the last three decades [2]. Interestingly, obesity is also an increasing and highly prevalent condition [3]. Several lines of evidence point to a reciprocal link between depression and obesity. It has also been suggested that obesity and depression may share common background; however, the precise mechanisms for this association are not yet fully understood [4]. As described in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), one of the criteria for the diagnosis of MDD may include significant weight loss/gain or decrease/increase in appetite [5].

Contrary to other psychiatric (e.g. schizophrenia [6,7] or eating disorders [8-10]) and medical conditions (e.g. obesity [11,12] or Prader-Willi syndrome [13]), a survey of the literature reveals the amount of research exploring the neural correlates of appetite changes in the context of depressive episodes is surprisingly scarce and recent [14]. Few studies have explored the association of appetite brain activity in the context of depression [14-16]. Only one study [16] explored the patterns of brain activity in response to food stimuli in depressed patients with increased or decreased appetite and in controls. The other works [14,15] provide the first clues regarding the association of appetite neural correlates with endocrine, metabolic and inflammatory alterations in depressed patients.

Antidepressant drugs constitute the standard of care for major depression disorder and selective serotonin reuptake inhibitors (SSRI) are often used as the first-line of pharmacotherapy [17]. Evidence indicate that antidepressant treatment may induce weight gain by acting in central mechanisms regulating appetite and food intake [18]. Previous studies show that weight gain associated with antidepressants may reflect their action on monoamine pathways, which include serotonergic receptors, among others [4]. Furthermore, serotonin plays an important role in eating behavior and preference for certain macronutrients, such as carbohydrates [19]. Paroxetine is a well-tolerated, high affinity and potent SSRI that is effective in the treatment of both depressive and anxiety disorders across the age range [20]. Despite being well-tolerated, paroxetine treatment induces more weight gain than other SSRIs [21]. Interestingly, paroxetine, has been associated with a marginal weight loss during acute treatment but associated with significant weight gain when used over longer periods [18]. Several other antidepressants have also been associated with weight gain; yet, the underlying mechanisms that link antidepressant use and weight gain are not fully understood [4].

While for schizophrenia [7,22] and obesity [23] there are studies exploring the impact of pharmacological treatment in appetite related neurocircuitry, to the best of our knowledge, no study has been published exploring this question on depressed patients. Depressed patients often present alterations in weight and appetite. Moreover, antidepressant treatment with paroxetine may also induce body weight alterations possible through changes in appetite and eating behavior. Therefore, it is important to understand the impact of this antidepressant on brain appetite processing system. Here we explore the neural correlates of depression and antidepressant treatment with paroxetine in neural processing of appetite information.

SUBJECTS AND METHODS

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by local ethics review boards. The goals and nature of the tests were explained to potential participants and all volunteers provided informed written consent.

Study sample

Subjects

Figure 1A shows the flow diagram of the recruitment process. The patients who enrolled in the present study (n=23) were recruited from the emergency room at Hospital de Braga, diagnosed with major depressive disorder, identified as treatment naïve and the treatment prescription was paroxetine 20 mg. Exclusion criteria included inability to understand informed consent, incapacity and/or inability to attend the evaluation/assessment session(s), diagnosed neuropsychiatric disorder, age under 18 years old and inability to perform the MRI acquisition. The first evaluation session (M1) occurred in the next day of the diagnose. As depicted in figure 1B evaluation session occurred during the afternoon, after 2 hours fasting period patients were administered with paroxetine 20 mg and started the anthropometric and psychological assessment. 1 hour after the administration of paroxetine patients performed a functional magnetic resonance imaging session in conjugation with a task that required visual processing of appetitive stimuli. After 6 to 8 weeks of treatment with daily paroxetine 20mg patients were reassessed

using the same experimental protocol (M2). 4 patients did not wish to participate in the second evaluation session; therefore, the number of participants at M2 was n=19. In addition, 16 healthy subjects were enrolled and perform the same experimental protocol to serve as control, yet instead of paroxetine 20 mg a placebo was administered. A demographic and clinical evaluation was performed in each assessment and gender, age and education (years of formal education) were recorded to be used as control variables.

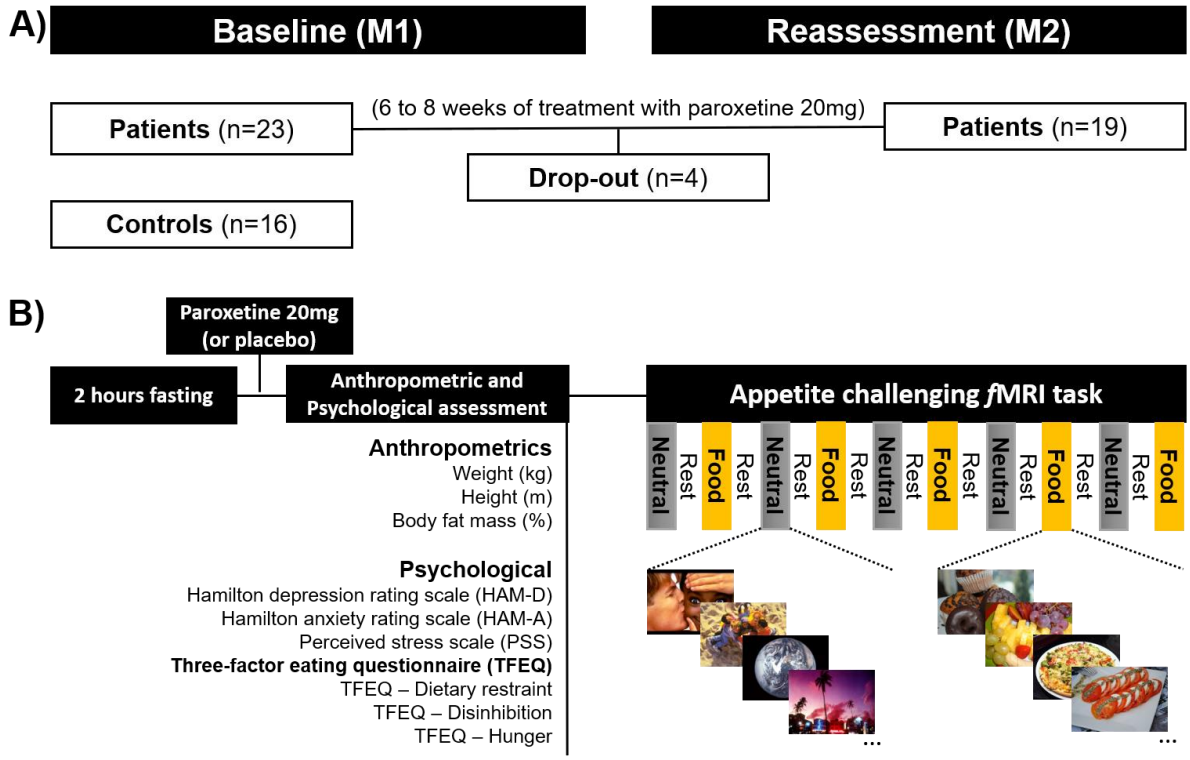

Figure 1 – Schematic representation of study design. A) participants selection (M1) and flow diagram of patient's reassessment (M2). **B)** Flow diagram of the evaluation session for every participant. Note that patients toke 20mg of paroxetine before each evaluation while controls toke a placebo.

Anthropometric assessment

Anthropometric measures included weight and relative body fat mass (%BF) (Tanita® BF 350 Body Composition Analyzer; Tanita Corporation, Tokyo, Japan), height (stadiometer Seca® 217; Seca GmBH & Co Kg, Hamburg, Germany) and waist circumference (WC; Seca® 201; Seca GmBH & Co Kg, Hamburg, Germany). Body mass index (BMI) was calculated as weight (Kg)/height $(m)^2$.

Psychological and eating behavior assessment

Anxiety and depressive symptomatology and perceived stress were assessed for psychological characterization using validated instruments. Tests were applied at each assessment by an experienced psychologist. Briefly, depressive symptomatology was assessed using the Hamilton depression rating scale (HAM-D) [24], anxiety symptomatology with the Hamilton anxiety rating scale (HAM-A) [25] and perceived stress with the Perceived stress scale (PSS) [26]. Eating behavior was assessed using the three-factor eating questionnaire in the dimensions of dietary restraint, disinhibition and hunger [27].

Statistical Analysis

Data is presented in median (Md) and interquartile rang (IQR) for continuous variables and in frequency (n) and percentage (%) for categorical variables. Differences in anthropometric, psychological and eating behavior between controls and patients (either at M1 and M2) were assessed using the Wilcoxon–Mann–Whitney test, and difference within patients at M1 and M2 were assessed sing the Wilcoxon Signed Ranks Test. Statistical analysis was conducted using the IBM SPSS Statistics v25 and statistical significance was defined at $p < .05$ level.

MRI data acquisition, pre-processing and analysis of imaging data

fMRI Data Acquisition

Each participant was scanned on a clinical approved 1.5 T Siemens Magnetom Avanto system (Siemens Medical Solutions, Germany) using a 12-channel receive-only head array coil. For the functional acquisition, a T2* weighted echo-planar imaging acquisition was acquired: 38 interleaved axial slices, repetition time 2500 ms, echo time 30 ms, field of view 224 mm × 224 mm, flip angle 90°, in-plane resolution 3.5 mm × 3.5 mm, slice thickness 3.5 mm, and between-slice gap 0.5 mm. To optimize the sensitivity in the orbitofrontal cortex, a tilted acquisition in an oblique orientation of 30° relative to the anterior-posterior commissure line was used. In total, 190 volumes were acquired during the task. The task stimulus was presented using the fully integrated fMRI system IFIS-SA (Invivo Corporation, United States) and the same system was used to record the subject key-press responses. One high-resolution T1-weighted Magnetization-Prepared Rapid Acquisition with Gradient Echo sequence, with 1 mm × 1 mm × 1 mm voxel size, repetition time 2.73 s, echo time 3.48 ms, flip angle 7°, field of view 234 mm × 234

mm, and 176 slices was acquired. This anatomical sequence was used to support the preprocessing of the functional data and project the functional maps.

fMRI Task

A standard period of 3h between the last meal and the beginning of the fMRI task was used. As show in figure 1B participants underwent a passive viewing task with two experimental conditions: neutral and food. 10 blocks of 11 pictures were presented, with 5 blocks in the neutral condition and 5 blocks in the food condition. The pictures of the neutral condition were selected from the International Affective Picture System (IAPS) and the pictures from the food condition were selected from the Open Library of Affective Foods (OLAF). Pictures with median values of valence, arousal, and dominance were selected from IAPS; then, images matched for visual complexity were selected from the OLAF with identical values of rating as the ones selected from IAPS. Each block lasted for 30.2 s and was separated by others by resting periods of 18.0 s during which participants watched a black screen with a white plus signal. This passive viewing task was used to avoid recruiting other cognitive process in conjugation with the presentation of the stimuli.

fMRI Data Preprocessing

The functional scans from each participant were preprocessed using the Statistical Parametric Mapping (SPM) version 12 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, United Kingdom) using MATLAB version R2018a (The MathWorks Inc., United States). The preprocessing procedures included: slice-timing correction using the first slice as reference; realignment to the mean volume of the acquisition; nonlinear spatial normalization to Montreal Neurological Institute (MNI) standard space and resampling to 2 mm × 2 mm × 2 mm voxel size; spatial smoothing with a 8 mm full-width at half-maximum Gaussian kernel; high pass temporal filtering at 128 s. Participants with more than 2 mm of movement (1 voxel) were excluded (n = 0).

fMRI Data Analysis

All models were implemented using the Statistical Parametric Mapping (SPM) version 12 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, United Kingdom) using MATLAB version R2018a (The MathWorks Inc., United States). For the first-level analysis, one general linear model (GLM) was computed per participant. For this GLM, the regressors of interest included: the type of stimulus (neutral and food) and 6 motion parameters estimated during the realignment step. The onset and duration of the regressors were defined accordingly to the stimulus represented in with a boxcar function and the regressors were convolved with the canonical hemodynamic response function.

At the group level (second-level analysis), the analysis was performed using several statistical designs all of them controlled for age and gender. Differences in BOLD signal between controls and participants at M1 and M2 were analyzed using an independent sample t-test, BOLD signal differences within patients from M1 to M2 were assessed using a paired sample t-test. In addition, the activated clusters obtained as a result of this contrast were defined as regions of interest (ROIs) and further analyses were performed separately for each of them by taking into account the estimation of the BOLD signal change averaged over all voxels in the ROI using the SPSS software and statistical significance was defined at $p < .05$ level. Specifically, we tested the correlation [non-parametric – Kendall's tau (r_τ)] of the change in BOLD signal with BMI, %BF, HAM-D, HAM-A, PSS, TFEQ-dietary restraint, TFEQ-disinhibition and TFEQ-hunger at M1 and M2 and the difference between both. Results were considered statistically significant after correcting for multiple comparisons using cluster correction (minimum cluster size can be obtained in the table corresponding to the analysis). The minimum cluster size was determined with 3DClustSim (AFNI version 17.0.13; National Institute of Mental Health). This program determines a minimum cluster size with Monte Carlo Simulation to achieve a corrected significance of $p < 0.05$ with an initial voxel-wise threshold of $p < 0.005$.

RESULTS

Sample characterization

Table 1 shows the characterization of controls and patients at M1 and M2. Approximately 70% of the participants were females. Despite of an 11 years difference in the median age between controls and patients, no statistically significant difference in age was detected. Controls were significantly more educated than participants. No significant differences in BMI or body fat were observed between control and patients and from M1 to M2.

Table 1 – Participants characterization at baseline (M1) and reassessment (M2).

	Controls n=16	**Patients M1** n=23	**Patients M2** n=19	**Controls *vs* Patients M1** ¥ ***p***	**Controls *vs* Patients M2** ¥ ***p***	**Patients M1 *vs* Patients M2** † ***p***
Sociodemographic						
Females (n; %)	11; 68.8	17; 73.9	14; 73.7	–	–	–
Age (years) (Md; IQR)	26; 8.75	37; 28	37; 24	.173	.314	–
Education (years) (Md; IQR)	16.5; 4.75	12; 10.5	12; 10	**.036**	**.042**	–
BMI (kg/m^2) (Md; IQR)	24.07; 9.01	25.36; 8.49	25.57; 7.02	.851	.741	.708
Body fat (%) (Md; IQR)	24.6; 8.05	33.4; 10.7	32.1; 10.6	.060	.105	.319
Psychological						
HAM-D (Md; IQR)	0; 1.25	22; 12.5	10; 12	**<.001**	**<.001**	**<.001**
HAM-A (Md; IQR)	1; 2	23; 14	15; 19	**<.001**	**<.001**	**<.001**
PSS (Md; IQR)	10.5; 11.25	28; 8	20; 9	**<.001**	**<.001**	**<.001**
Eating behavior (TFEQ)						
Dietary restraint (Md; IQR)	11; 7.25	12; 8	7; 9	.958	.590	**.035**
Disinhibition (Md; IQR)	4.5; 3.75	5; 3.5	6; 4	.615	.566	.833
Hunger (Md; IQR)	3.5; 3.5	3; 4.5	3; 6	.889	.964	.354

Data is presented in absolute frequency (n) and relative frequency (%) or in median (Md) and interquartile rang (IQR). ¥ Wilcoxon–Mann–Whitney test. † Wilcoxon Signed Ranks Test.

HAM-D, HAM-A and PSS scores were significantly higher in patients at M1 than in controls. After the 6 to 8 weeks of treatment with paroxetine 20 mg there was a significant reduction in the scores of all the psychological variables that were assessed; however, patients at M2 continued to present a significantly higher psychological morbidity than controls.

No significant differences between controls and patients (either at M1 and M2) were observed in the dimensions of eating behavior assessed by the TFEQ; yet, the treatment period resulted in a significant decrease in the dietary restraint of patients.

Brain activity related to appetite information processing in patients and controls

Table 2 and Figure 2 shows the brain regions with significant differences in the BOLD signal response between patients and controls when visualizing pictures of food versus neutral pictures. Compared to controls, patients presented a significantly lower brain activation in three clusters that included the right (inferior temporal gyrus and superior temporal gyrus) and left (middle temporal gyrus and inferior temporal gyrus) temporal lobe and the right cerebellum (Crus 1 and 2). No brain regions displayed statistically significant higher activation in patients compared to controls. Interestingly, when comparing controls with patients at M2 no brain regions presented statically significant differences regarding BOLD signal response.

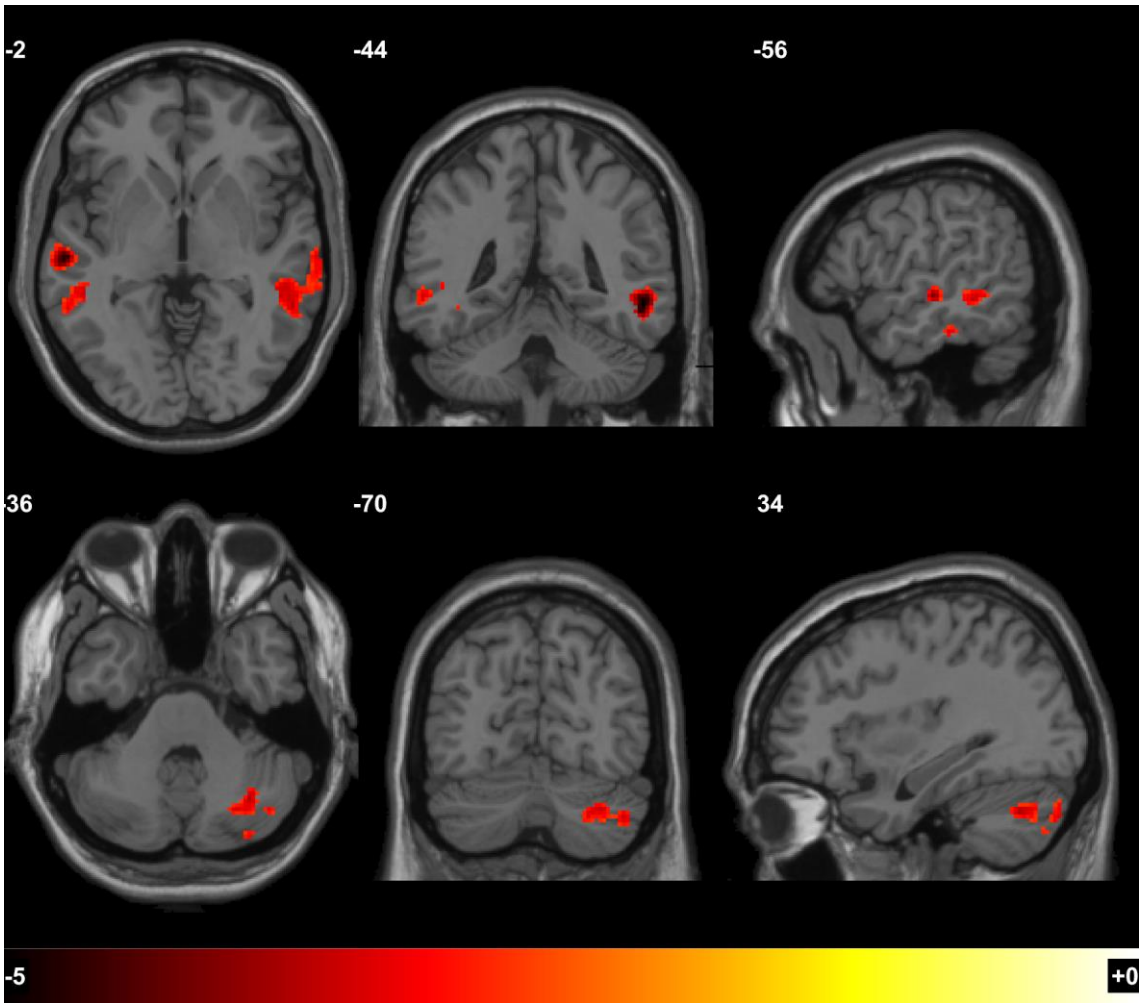

Figure 2 – Brain regions in which the BOLD signal when presented images of food (*versus* neutral images) was significantly different between controls and patients at M1. In each row of slices numbers represent the Montreal Neurological Institute (MNI) coordinates in the inferior-superior, anterior-posterior, and left-right dimensions, respectively.

Table 2 – Brain regions in which patients presented a significantly decreased BOLD signal compared to controls when visualizing images of food.

Patients < Controls (Food > Neutral)			MNI Coordinates		
Region Label	**Cluster extent**	**t-value**	**x**	**y**	**z**
Right Inferior Temporal Gyrus	502	-4.975	54	-44	-8
Right Superior Temporal Gyrus		-4.421	68	-26	4
Left Middle Temporal Gyrus	220	-4.766	-60	-20	-4
Left Inferior Temporal Gyrus		-3.354	-56	-28	-22
Right Cerebellum (Crus 1)	218	-3.700	34	-64	-36
Right Cerebellum (Crus 2)		-3.348	34	-84	-38

Table shows all local maxima separated by more than 20 mm. Regions were automatically labeled using the AnatomyToolbox atlas. x, y, and z =Montreal Neurological Institute (MNI) coordinates in the left-right, anterior-posterior, and inferior-superior dimensions, respectively. ($t > 2.7238$; $p < 0.0050$; $df = 35$; minimum extent = 158)

Change in brain activity related to appetite information processing after treatment

Table 3 and Figure 3 show the brain regions with significant differences in the BOLD signal response between M1 and M2 in patients when visualizing pictures of food versus neutral pictures. After the six to 8 weeks of treatment with Paroxetine 20 mg, patients displayed significantly higher brain activation in a cluster located at the left inferior frontal gyrus (pars orbitalis and pars triangularis), in a cluster located at the left middle frontal gyrus and the left superior frontal gyrus, a cluster located in the left anterior cingulated cortex and in a cluster located in the right insula lobe. No brain regions displayed statistically significant lower activation in patients at M2 compared to M1.

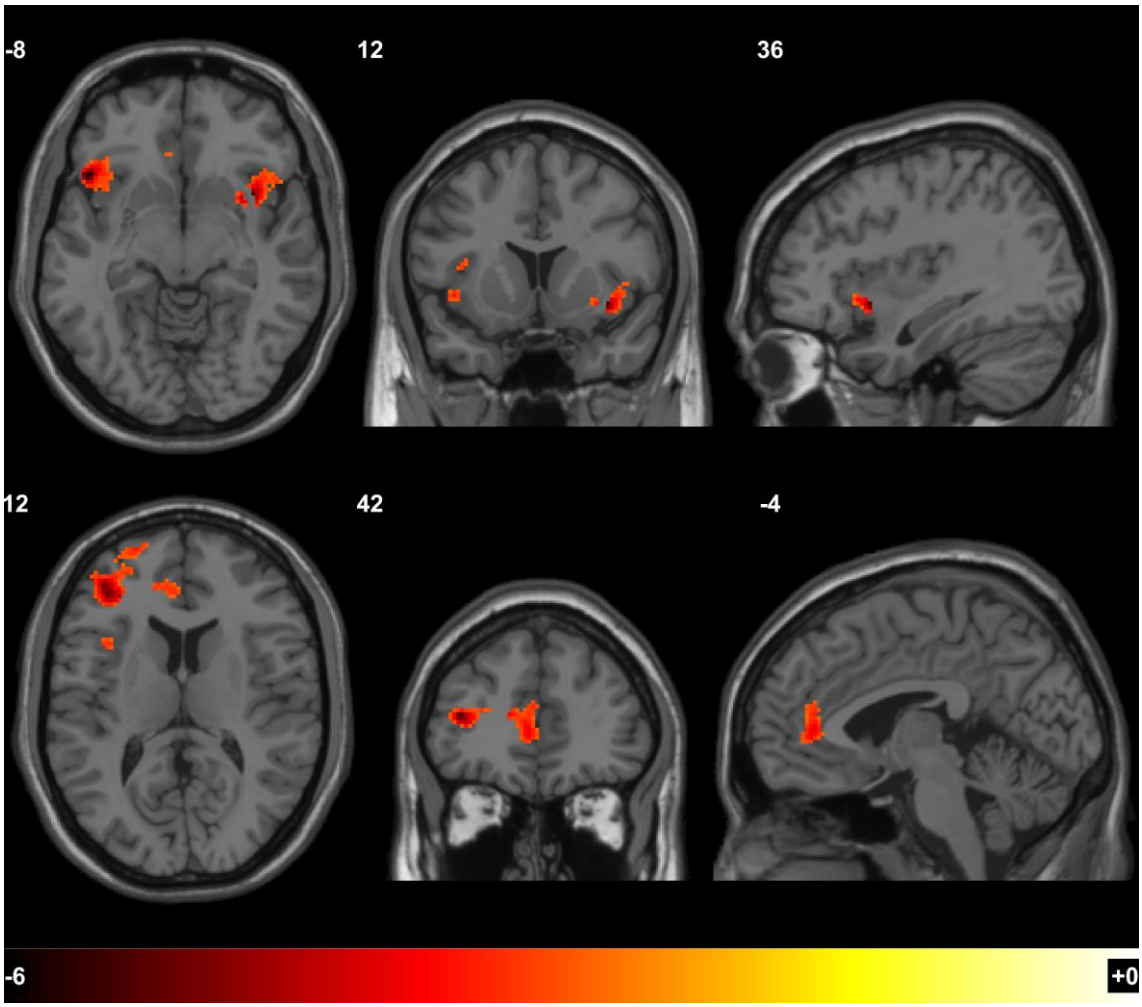

Figure 3 – Patients brain regions in which the BOLD signal response to images of food (*versus* neutral images) was significantly different at M2 compared to M1. In each row of slices numbers represent the Montreal Neurological Institute (MNI) coordinates in the inferior-superior, anterior-posterior, and left-right dimensions, respectively.

Table 3 – Brain regions in which patients presented a significantly increased BOLD signal after treatment when visualizing images of food.

Patients M1 < Patients M2 (Food > Neutral)			**MNI Coordinates**		
Region Label	**Cluster extent**	**t-value**	**x**	**y**	**z**
Left Inferior Frontal Gyrus (pars Orbitalis)	340	-5.833	-48	22	-6
Left Inferior Frontal Gyrus (pars Triangularis)		-3.537	-40	16	16
Left Middle Frontal Gyrus	369	-5.309	-38	42	14
Left Superior Frontal Gyrus		-4.287	-22	62	8
Right Insula Lobe	219	-5.236	36	12	-8
Left Anterior Cingulate Cortex	185	-3.932	-4	40	6

Table shows all local maxima separated by more than 20 mm. Regions were automatically labeled using the AnatomyToolbox atlas. x, y, and z =Montreal Neurological Institute (MNI) coordinates in the left-right, anterior-posterior, and inferior-superior dimensions, respectively. ($t > 2.8982$; $p < .005$; df = 17; minimum extent = 168)

Correlation between anthropometrics, psychological variables and eating behavior with brain activity change with treatment.

As previously mentioned, treatment with paroxetine resulted in an increase in brain activity in four clusters. We then treated these clusters as ROIs and correlated the parameters estimates change (beta change) from M1 to M2 with anthropometrics, psychological variables and eating behavior (Figure 4).

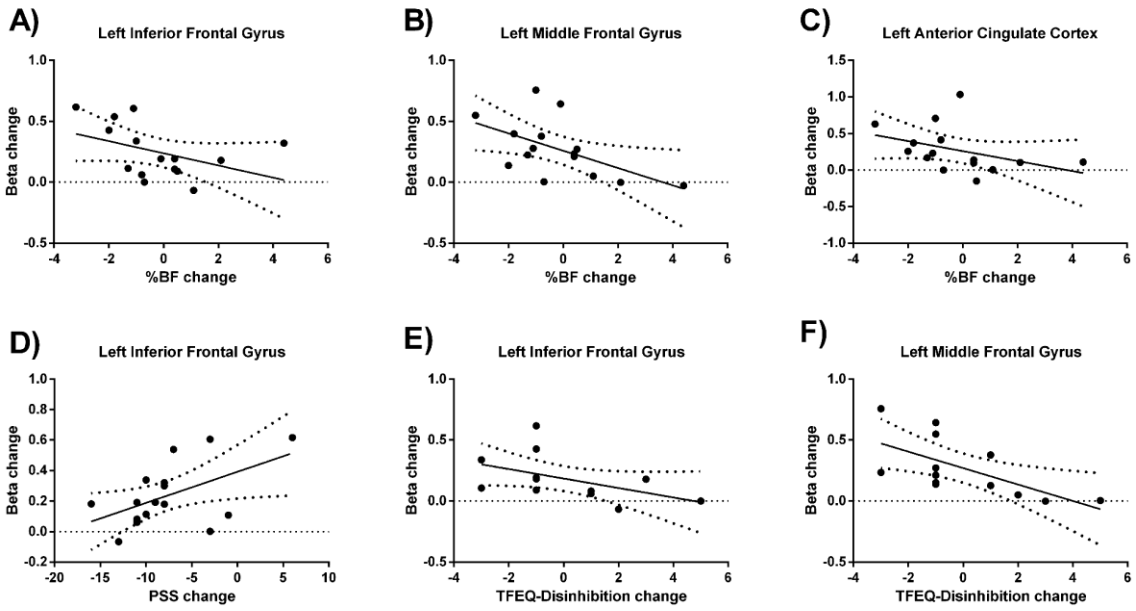

Figure 4 – Correlation between brain activity change after treatment and changes in anthropometrics, psychological variables and eating behavior. A) Significant correlation between brain activation change in left inferior frontal gyrus and change in %BF after treatment. **B)** Significant correlation between brain activation change in left middle frontal gyrus and change in %BF after treatment. **C)** Significant correlation between brain activation change in left anterior cingulate cortex and change in %BF after treatment. **D)** Significant correlation between brain activation change in left inferior frontal gyrus and change in PSS after treatment. **E)** Significant correlation between brain activation change in left inferior frontal gyrus and change in TFEQ-Disinhibition after treatment. **F)** Significant correlation between brain activation change in left middle frontal gyrus and change in TFEQ-Disinhibition after treatment. (Full line represents the linear regression equation and doted lines represent the 95% confidence intervals).

Changes in %BF were negatively correlated with parameters estimates changes in the cluster located in the left inferior frontal gyrus (r_τ = -.390; p < .05), at the cluster located at the left middle frontal gyrus (r_τ = -.429; p < .05) and at the left anterior cingulate cortex (r_τ = -.390; p < .05).

The variation in perceived stress (PSS) from M1 to M2 was positively associated with parameters estimates changes in the cluster located in the left inferior frontal gyrus (r_τ = .350; p < .05). Negative correlation between the changes in TFEQ-Disinhibition that occurred during the treatment period and parameters estimates changes in the cluster located in the left inferior frontal gyrus (r_τ = -.402; p < .05), at the cluster located at the left middle frontal gyrus (r_τ = -.529; p < .05) were also observed. No other statistically significant correlations between changes in interest variables and parameters estimates changes was observed.

Supplementary material shows the, age and gender controlled, associations of brain activation in response to food stimulus with psychological variables and eating behavior in controls, patients at M1 and patients at M2 independently. Supplementary Table 1 and Supplementary Figure 1 show the associations of the brain activation in response to the food stimulus with HAM-D scores in patients and Supplementary Table 2 and Supplementary Figure 2 show the associations of the brain activation in response to the food stimulus with HAM-A scores. No significant associations of PSS, TFEQ-dietary restraint, TFEQ-disinhibition and TFEQ-hunger with brain activation in response to the food stimulus were observed for patients at M1 or M2.

DISCUSSION

In the present work, we investigated the neural appetite information processing, anthropometric, psychological and eating behavior changes in treatment naïve depressed patients after a short period of paroxetine treatment.

Over a short period of time (6 to 8 weeks) we observe a significant reduction in the scores of depressive mood, anxiety symptomatology and perceived stress; yet, these decreases were not sufficient to bring patients to the same levels as controls. From M1 to M2 patients presented a decrease of 12 points in the HAM-D median score that is the range of improvement observed in the clinical trial of paroxetine submitted to the Food and Drug administration [28]. For the HAM-A we observe a decrease of 8 points in

the median score from M1 to M2 that is slightly lower than the values of improvement reported for generalized anxiety disorder [29].

While no difference between controls and patients were observed for eating behavior, our results show that, when visualizing pictures of food, treatment naïve depressed patients present lower brain activation bilaterally in temporal cortex and in right cerebellum compared to controls. Simmons *et al.* [16] found group differences in the insular regions when exploring the neural correlates of appetite in depressed patients and controls. In a later study that explored endocrine, metabolic and immune states in the same groups, Simmons *et al.* [14] reported group differences in the right putamen, right parahippocampal gyrus, right occipital lobe, right dorsal midinsula, right posterior insula, the left ventral striatum, and the ventral tegmental area. Unlike our study, the mentioned works explored the differences in brain activation between 3 distinct groups (depressed patients with increased or decreased appetite and controls) while in our work most of the patients reported no changes in appetite or appetite decrease.

Examining reports of the neural correlates of the contrast between viewing food and nonfood pictures in other context may be helpful for the understanding of our results. In a meta-analysis van der Laan *et al.* [30] found that bilateral posterior fusiform gyrus, the left lateral orbitofrontal cortex and the left middle insula activations where the most commonly activated brain regions in response to food pictures. Not surprisingly, the areas here reported are not the same, since we explore the differences between non-depressed and depressed subjects. Yet, several studies had implied the involvement of temporal areas as components of a network (temporo-insulo-opercular and orbitofrontal areas) involved in the processing of food stimuli [31]. Not so commonly reported, we found a decrease in the brain activity in the right cerebellum of depressed patients. Cerebellum is more than a motor coordination area and several lines of evidence suggest that it is also involved in the regulation of various functions including feeding control and behavior [32]. Cerebellum was found to present higher activation when visualizing high *versus* low energy content foods [30]. In a study that explored the cognitive control of appetite in normal weight and morbid obese subjects, bilateral activation of cerebellum was observed when contrasting the inhibition condition, where participants had to inhibit urges to eat the food, with the passive viewing condition and when contrasting imaginary eating with passive viewing [11]. Assuming the involvement of the brain areas here reported in the appetite processing and regulations, our results indicate that treatment naïve depressed patients present a dysregulation of brain activity that can be a cause, or a consequence of the appetite dysregulation observed in depression.

Furthermore, we observe that 6 to 8 weeks of daily paroxetine treatment resulted in an increase in brain activity in left frontal areas (lateral prefrontal cortex), in the left anterior cingulate cortex and in the right insula. Several works have shown increased activation of prefrontal cortex [11,30,33-37], anterior cingulate cortex [11,35,38,39] and insula [11,33,34,39-41] when visualizing pictures of food. Interestingly, in the work of Simmons *et al.* [16] non-treated depressed patients with increased appetite presented increased activation in response to food stimuli in right anterior insula and in left posterior orbitofrontal cortex compared to non-treated depressed patients with decreased appetite and controls. Taken together, our results indicate that paroxetine treatment impact on appetite-related processing regions.

Paroxetine has been associated with a marginal, but statistical significant, weight loss during acute treatment while associated with significant weight gain when used over longer periods [18]. In the present work, no significant differences were observed for BMI or %BF between control and patients or over the treatment period. Despite of the absence of significant results, with our design, we cannot rule out an effect of paroxetine on body composition. The possibility of our observations had coincided with the transition between weight loss and weight gain can possibly explain the absence of differences here reported. Supporting this possible explanation are the positive and negative changes observed in %BF easily observed in Figure 4. Also, the changes in %BF were negatively associated with the changes in brain activity in left lateral prefrontal areas and in the left anterior cingulate cortex. In the same line of our results, Brooks *et al.* [42] found a decrease in the activation of the dorsolateral prefrontal cortex in response to food stimuli in obese individuals compared to normal weight subjects. Also, Tuulari *et al.* [11] contrasting inhibition minus imaginary eating comparison, subjects showed a stronger activations in anterior cingulate cortex in normal-weight than obese subjects. These results may suggest that individuals that presented a decrease in %BF during the 6 to 8 weeks of paroxetine treatment have an enhanced activation in inhibitory control (anterior cingulate cortex) and internal awareness (prefrontal cortex) brain regions [23,43].

No significant differences were observed regarding eating behavior between controls or patients; nevertheless, paroxetine treatment was able to promote a significant reduction on the score of the TFEQ-dietary restraint. It was previously reported [44] that, comparing with a normative population, patients with mood disorders under psychiatric medication presented a higher TFEQ-disinhibition score. The author also found a positive correlation between HAM-D score and the TFEQ-dietary restraint score among patients with mood disorders. Despite not comparable with our results, that report highlight the

association between psychiatric medication and eating behavior. Further supporting this notion, it was previously shown that antidepressant users present a higher caloric intake than non-users [45,46].

We observed that, after the treatment with paroxetine, a significant negative correlation between the change in brain activity in left inferior and middle frontal gyrus and the change in the TFEQ-disinhibition score was present. In a study of baseline brain activity, Zhao *et al.* [47] reported that prefrontal areas, including the left middle frontal gyrus, were positively associated with the eating behavior dimensions assessed by TFEQ. Lateral prefrontal areas have been reported to be involved in food motivation, reward sensitivity, and impulsivity [48], which support the finding observed in these areas. Disinhibition is defined as a tendency to overeat in the presence of palatable foods; yet, other stimuli, such as stress, sadness or depression may also be involved [27]. Taken together our results, paroxetine treatment seems to be involved in changes in eating behavior in depressed patients and in the associated brain activity.

Interestingly, while no association was observed for the change in HAM-D and HAM-A, the change in PSS score was positively correlated with the change in brain activity in the left inferior frontal gyrus. The role of stress in eating behavior widely recognized [49-53]. In a study that enrolled normal weight healthy subjects, acute stress was associated with decreased brain response to food cues in the orbitofrontal cortex among other areas [50]. In this work, a smaller change in the brain activation in the left inferior frontal gyrus is observed in the patients that presented a reduction in the perceived stress. As mentioned previously, the left lateral orbitofrontal cortex activation is one of the most commonly reported brain regions in response to food pictures [30]. This may suggest an evolvement of the left inferior frontal gyrus in the appetitive "stress" eating [53].

Some strengths and limitations of this work deserve to be mentioned. In the present work we studied the neural correlates of appetite in treatment-naïve depressed patients and the changes in appetite brain activity as result of a pharmacological treatment for depression. The recruitment of treatment naïve patients allowed us to study the appetite-associated brain alterations without the possible influence of previous medications. The specificity of the patients that were enrolled led to a relatively low sample sizes, which may decrease the likelihood of detecting less robust group differences or associations. Simmons *et al.* [16] have previously explored the neural correlates of appetite in untreated patients with depression and increased or decreased appetite. Our study differentiates from that using treatment-naïve depressed patients. In our sample most of the patients presented appetite or weight decrease or no changes in appetite; therefore, we did not divide them into groups. This is an ongoing study and we expect to increase

our sample size. Another limitation that we expect to overcome in the future is the short duration of the study since we plan to assess these patients also at 6, 12 and 24 months after the beginning of treatment.

In summary, to the best of our knowledge, here we explore for the first time the differences in brain activity in response to food stimuli between treatment-naïve depressed patients and healthy controls. We observed that depressed patients present a decrease brain activation in areas associated with the processing of food stimuli (temporal cortex) and areas that integrate somatic responses to food (cerebellum). Furthermore, we explore for the first time the impact of an SSRI in brain activity associated to appetite processing during treatment of depression. Areas associated with reward (lateral prefrontal cortex), decision-making (anterior cingulate cortex) and integration of sensory cues (insula) present a higher activation after 6 to 8 weeks of treatment with paroxetine. We also show that the difference in brain activity after treatment are associated with body composition, eating behavior and stress. Our work is relevant in the sense that identifying the potential mechanism by which depression and antidepressant medication increases or decrease appetite, and subsequently the risk of obesity or underweight, can help the development of strategies to prevent associated comorbidities.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Contributions

CPN and JMB performed the statistical analysis. CPN, JR, RV, SF, AC, NS and JMB contributed to the data analyses and discussion. JMB recruited the participants. CPN, JR, RV, SF and RM collected the data. CPN wrote the first draft of the manuscript. JMB and conceived and designed the study. All the authors revised the manuscript. JMB had access to all the data in the study.

Acknowledgments

This work was funded by the Portuguese Foundation for Science and Technology (FCT) under the project PTDC/DTP-PIC/6936/2014. C.P.N. was supported by the Portuguese Science Foundation (FCT) doctoral scholarship PD/BD/106050/2015 via Inter-University PhD Programme in Ageing and Chronic Diseases. J.R. was supported by the FCT fellowship grant with the reference PDE/BDE/113602/2015 from the PhDiHES program. R.V. was supported by a scholarship from the project PTDC/DTP-PIC/6936/2014 (FCT; UMINHO/BI/340/2018). S.F. was supported by a combined Ph.D. scholarship from the FCT and the company iCognitus4ALL – IT Solutions, Lda (PD/BDE/127839/2016) from the PhDiHES program. A.C. was supported by a scholarship from the project NORTE-08-5639-FSE-000041 (NORTE 2020; UMINHO/BD/51/2017). R.M. was supported by the FCT fellowship grant with the reference PDE/BDE/113604/2015 from the PhDiHES program.

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SUPPLEMENTARY MATERIAL

Association of brain appetite processing activity and psychological variables and eating behavior

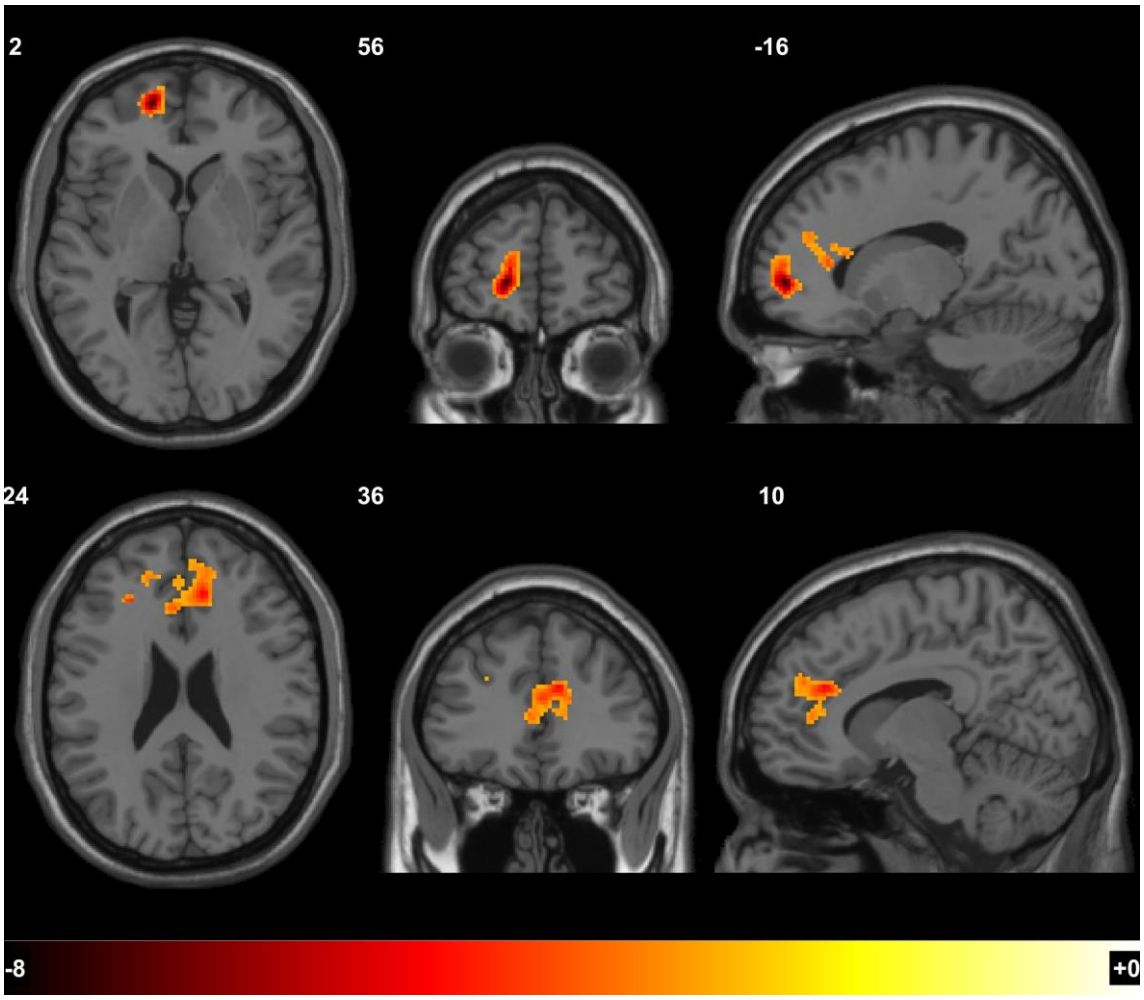
Multiple regression, controlled to age and gender, were used to test these associations of brain activation in response to food stimulus with psychological variables and eating behavior in controls, patients at M1 and patients at M2 independently.

Supplementary Table 1 and Supplementary Figure 1 show the associations of the brain activation in response to the food stimulus with HAM-D scores in patients. No association of the HAM-D scores with brain activation in response to food stimulus was observed for patients at M1. However, patients at M2, presented a negative association between the HAM-D scores and brain activation in response to food stimulus in the left superior orbital gyrus (r = -.799; $p < .001$) and in the anterior cingulate cortex (left and right) (r = -.731; $p = .001$). Taken together, these results indicate that, in patients, after the 6 to 8 weeks of treatment with paroxetine 20 mg, a decreased brain activation in these regions was correlated with higher depressive symptomatology.

Supplementary Table 1 – Brain regions in which BOLD signal response when visualizing images of food was significantly associated with the scores in the HAM-D.

HAM-D (Food > Neutral)			MNI Coordinates		
Region Label	Extent	t-value	x	y	z
Patients M1					
No statistically significant regions	—	—	—	—	—
Patients M2 (negative)					
Left Superior Orbital Gyrus	274	-7.355	-16	56	2
Right Anterior Cingulate Cortex	600	-4.970	10	36	24
Left Anterior Cingulate Cortex		-4.075	-4	38	8

Table shows all local maxima separated by more than 20 mm. Regions were automatically labeled using the AnatomyToolbox atlas. x, y, and z =Montreal Neurological Institute (MNI) coordinates in the left-right, anterior-posterior, and inferior-superior dimensions, respectively (Patients M2 - t > 3.0545; $p < .005$; df = 12; minimum extent = 179)



Supplementary Figure 1 – Brain regions in which BOLD signal response to images of food (*versus* neutral images) was significantly associated with the scores in the HAM-D in patients at M2. In each row of slices numbers represent the Montreal Neurological Institute (MNI) coordinates in the inferior-superior, anterior-posterior, and left-right dimensions, respectively.

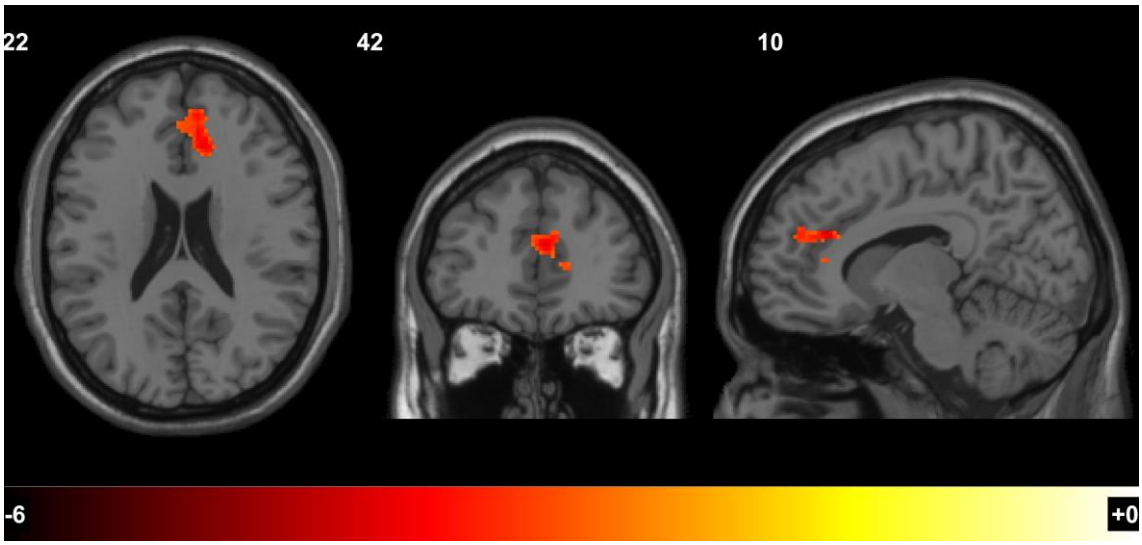
Supplementary Table 2 and Supplementary Figure 2 show the associations of the brain activation in response to the food stimulus with HAM-A scores. Similar to the results observed for HAM-D, patients at M2, presented a negative association between the HAM-A scores and brain activation in response to food stimulus in the anterior cingulate cortex (right hemisphere) (r = -.705; p = .002).

No significant associations of PSS, TFEQ-dietary restraint, TFEQ-disinhibition and TFEQ-hunger with brain activation in response to the food stimulus were observed for patients at M1 or M2.

Supplementary Table 2 – Brain regions in which BOLD signal response when visualizing images of food was significantly associated with the scores in the HAM-A.

HAM-A (Food > Neutral) Region Label	Extent	t-value	MNI Coordinates x	y	z
Patients M1					
No statistically significant regions	—	—	—	—	—
Patients M2 (negative)					
Right Anterior Cingulate Cortex	200	-4.112	10	34	24

Table shows all local maxima separated by more than 20 mm. Regions were automatically labeled using the AnatomyToolbox atlas. x, y, and z =Montreal Neurological Institute (MNI) coordinates in the left-right, anterior-posterior, and inferior-superior dimensions, respectively. (Controls - t > 3.0545; $p < .005$; df = 12; minimum extent = 184; Patients M2 - t > 3.0545; $p < .005$; df = 12; minimum extent = 179)



Supplementary Figure 2 – Brain regions in which BOLD signal response to images of food (*versus* neutral images) was significantly associated with the scores in the HAM-A in patients at M2. In each row of slices numbers represent the Montreal Neurological Institute (MNI) coordinates in the inferior-superior, anterior-posterior, and left-right dimensions, respectively.

CHAPTER VI

General discussion, conclusion and future perspectives

GENERAL DISCUSSION

The relationship between metabolism and mood is well recognized and known for a long time. While several works have focused on specific aspects of this intricated association, in this work we explore numerous facets of this problematic by comprehensively characterizing the relationship between metabolism and mood in health and in disease.

In **Chapter I** we gathered the information from relevant literature regarding the prevalence of major depressive disorders and depressive mood, obesity and metabolic syndrome. We further addressed the association between mood and metabolism and the potential involved mechanisms. Considering the knowledge obtained from the scientific literature, we highlighted some of the knowledge gaps and shortcomings regarding the subject under analysis and, considering those, we defined the main objectives and research aims to be addressed on this work.

Several lines of evidence point to a positive bidirectional association of overweight and obesity with depressed mood in younger to middle age adults [1-3]. Interestingly, in older ages, works exploring this association provided conflicting results with some supporting the hypotheses that higher adiposity is associated with less depressive mood [4-8], and others indicating a positive association of adiposity and depressive mood [9-14]. Furthermore, the aging process is associated with changes in body composition that are characterized by a reduction in muscle mass and an increase in adiposity in the intraabdominal compartment [15,16]. Taking this into account, in **Chapter II** we explored the role of age in the association between mood and general and abdominal adiposity in older adults. We further investigated the influence of age on the association of the different abdominal adipose tissue compartments (subcutaneous adipose tissue - SAT and visceral adipose tissue - VAT) using magnetic resonance imaging techniques (MRI). Our analysis indicated that, in individuals with 50 or more years of age, the cross-sectional trend of the association between some adiposity indexes and mood depends on age. Adiposity measured by proxy indicators such as BMI, WC and SAT were positively associated with GDS score in the younger participants of our sample and the association became null or negative for the older ones. Importantly, VAT was positively associated with GDS independently of age. This last observation is particularly relevant in the sense that the results from the other adiposity indexes may suggest another "obesity paradox" that, as many others, may not be truth [17,18].

Excess of adiposity is associated with adverse health consequences [19,20]. Particularly, central obesity, that is defined as the excessive accumulation of fat in the visceral abdominal region, seems to be an important pathogenic fat depot [21,22]. It is also a central component of the constellation of metabolic abnormalities identified as MetS [23]. Systematic reviews and meta-analyses have previously shown a positive association between MetS and depressive mood [24-27]. This relationship was observed both in cross-sectional and longitudinal studies, and from the last ones there was evidence that the association was positive and bidirectional with baseline depression predicting the risk of developing MetS and baseline MetS predicting the risk of developing depression [25]. Considering the observation from **Chapter II** in which visceral adipose tissue was positively associated with depressive mood, we aimed to explore the association of mood with metabolic abnormalities (**Chapter III**). Despite the strong evidence that support the association between mood and metabolic syndrome, we were puzzled by aspects that were unexplored. First, MetS classification presents several limitations, such as including in the same group individuals with different metabolic profiles and differing levels of metabolic abnormality [28]. Also, the contribution of each of the components of the overall syndrome may differ while the classification systems attribute the same importance to all the components. Furthermore, the IDF classification for MetS excludes individuals who have metabolic risk factors but not central obesity [29]. These limitations led us to question if the association of MetS with mood is "dose-dependent", and if it is a result of the association between depressive mood with one or more of the MetS components. Second, MetS becomes more prevalent with the increase of age; however, the role of age in the association of MetS with depressive mood is unclear. Third, in order to reinforce the plausibility of a biological mechanisms underlying the association of MetS and depressive mood it should be reflected in features of the central nervous system associated with depressive mood.

In **Chapter III** we take advantage of a large cohort of individuals with 50 or more years of age to explore the questions above mentioned. To explore the separate contribution of each component to metabolic dysfunction we replicate the hierarchical four-factor structural equation model first proposed by Shen *et al.* [30,31] and later replicated by Levin *et al.* [32]. Using this strategy, we obtained factor scores for the latent variables in the model, namely metabolic dysfunction, obesity, glucose dysmetabolism, lipid imbalance and blood pressure. Metabolic dysfunction, glucose dysmetabolism and lipid imbalance were positively associated with GDS score, while obesity presented a U-shaped association and no association was observed for blood pressure. The trend of the relationship between metabolic dysfunction, obesity and

glucose dysmetabolism changed significantly with the age of the participants being positive in the younger participants and becoming negative in the older ones. Interestingly, the association of depressive mood and lipid imbalance was positive independently of age. This result was at the same time interesting and puzzling since, from the literature exploring the association between MetS components, studies addressing the relationship between blood lipids and depressive mood were the ones that provided more conflicting results [33-36]. The absence of association between the blood pressure factor and depressive mood across all the age range was also a surprising result since hypertension is one of the pillars that supports the vascular hypothesis of depression [37-39].

Still on **Chapter III**, we resort to a subsample to explore the mood and metabolic dysfunction relationship neural correlates. We focus on the functional connectivity of the default mode network, a resting state network well studied in the context of depressive symptomatology [40,41]. We observed that obesity could modify the association of GDS score with functional connectivity in the default mode network, and that the interaction of age with blood pressure also affect the association of GDS score with the functional connectivity in the default mode network. The investigation of several factors, such as the analysis performed in this work, might be critical for the explanation of the complex associations between mood, metabolism and age, and its impact in features of the central nervous system such as the functional connectivity of the brain networks.

Bariatric surgery is the best method of achieving substantial sustained weight loss in severely obese [42,43] and induces significant metabolism improvements [44]. At the same time, it also has been associated with improvements in mood, anxiety and cognition [45]. Therefore, bariatric surgery offers an important way to study the association between mood and cognition. In **Chapter IV** we investigate the temporal dynamics of the association of mood, anxiety, memory and executive function with metabolic biomarkers. Because metabolic biomarkers, anxiety, mood and cognition vary with the time that elapses from bariatric surgery, we hypothesized that that the relationship between those factors are not static but dynamic. We observed that over time the association of metabolic biomarkers with neurocognitive performance varied significantly, with association that become or ceased to be significant or even associations that changed the trend of the association rom negative to positive. Despite the need for replication, these results bring new perspectives to the study of the metabolism and neurocognitive performance relationship. Furthermore, some of the metabolic biomarkers that were analyzed are not well studied in the context of

mood, anxiety and cognition. Therefore, our work may not only contribute to knowledge regarding that associations but also to bring new questions that may help to advance the field.

Lastly, we explored the effects of treatment of major depressive disorder in body composition, eating behavior and appetitive neural correlates (**Chapter V**). Despite the involvement of appetite and weight changes on the clinical presentation of depression, the literature addressing the neural correlates of those aspects is surprisingly scarce. We observe that treatment-naïve depressed patients present decrease brain activation in areas associated with the processing of food stimuli (temporal cortex) and areas that integrate somatic responses to food (cerebellum). We also observed that areas associated with reward (lateral prefrontal cortex), decision-making (anterior cingulate cortex) and integration of sensory cues (insula) present a higher activation after 6 to 8 weeks of treatment with paroxetine and the differences in brain activity after treatment was associated with changes in body composition, eating behavior and perceived stress. An additional aspect of the eating process is the substantial hedonic reward value of food [46]. The neural correlates of the reward system and anhedonia in depression and antidepressant treatment are better characterized [47] and therefore may be important for the understanding of the results reported.

CONCLUSION

An important aspect of the work presented in this thesis is the exploration of age or time as a variable able to change associations that are usually observed as static. The ability of age to moderate the trend of the association between depressive mood and metabolic dysfunction and its components (including obesity) may be relevant to understand the inconsistent results observed in the literature but also to highlight the individuality of each person since the mentioned association is complex and may be affected by several factors.

An additional aspect of relevance in this work is the exploration of the effects of treatment both for obesity and subsequent metabolic complication associated but also the treatment for depression. The associations of mood and cognition are complex, and several systems may be involved. We found that several metabolic biomarkers are associated with mood anxiety and cognition and that association can change with time. While the involvement of insulin resistance has received attention as being involved in neurocognitive processes, here we show that metabolic markers, namely adipokines and incretins may

be also relevant in those processes. Closing the loop of treatment, we showed neural correlates of the appetite changes induced by depression and antidepressant treatment. These results may be potentially relevant to shed light in the route toward the development of new interventions targeting depression-associated appetite and weight changes and related complications.

Understanding the relationship between mood and metabolism can open new routes to target modifiable factors that can influence both mood and metabolic disturbance. In sum, although this work has contributed to an extension of the knowledge of the association between mood and metabolism, there is a long journey to follow.

FUTURE PERSPECTIVES

This thesis has taken steps toward the understanding of the relationship between mood disorders and metabolic disturbance; yet, several questions remain to be answered and others emerged from the results here presented.

As mentioned, exploring the neural correlates of the association between mood and metabolism is an important part of the characterization that needs to be performed. A step forward in this work would be to explore the correlates of the association in the central nervous system using multimodal MRI approach. Patients enrolled in the bariatric surgery program offer an invaluable opportunity to address this relationship, at least in the direction metabolism to mood. Unfortunately, during the experimental work of this thesis we could not perform MRI studies in these patients. Furthermore, we intend to explore the association of metabolic biomarkers with mood and appetite in the treatment-naïve depressed patients.

The works exploring the association of depressive symptoms with adiposity and metabolic disturbance are cross-sectional, which may be considered a limitation. Studies with longitudinal designs will be important to confirm our results. Our age range vary from 50 years to more than 80 years old, and a study assessing the changes over such a long period may not be possible; however, accelerated longitudinal designs may be appropriate to overcome that restraint.

Important aspects that were not tackled in the context of this work include the mechanisms and biological pathways that may be involved in the link of peripheral metabolic aspects and central aspects of mood and cognition. Therefore, experimental work in animal models is relevant for the manipulation of variables under study and to explore their mechanisms of action. Studies as the ones done by Kleinridders *et al.* [48]

with brain-specific knockout of insulin receptor (NIRKO) mice are essential to understand the mechanisms of action by which metabolism interact with mood and cognition. Furthermore, explore the brain distribution of receptors for metabolic hormones and molecules and if they are up- or down-regulated in metabolic disturbance and mood disorders is paramount for the full comprehension of such associations. For instance, GLP-1 and GIP receptors expression is decreased in hyperglycemia [49-51] and upregulated in the retina of streptozotocin-induced diabetic rats; however, the effect of metabolic and mood disturbances in the expression of brain GIP receptor are not known.

Finally, the design and development of intervention studies targeting modifiable factors identified in this work in order to ameliorate mood and metabolic disorders, would strengthen the importance of this interaction, constituting the perfect corollary for this thesis.

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