

of TO feeding has revealed a decrease in the amount of DAG and TAG almost to values noted in control rats. These changes were associated with strong decline in SCD1, FAS and ACC protein levels. Additionally, the level of carnitine palmitoyltransferase 1, a key enzyme involved in regulation of fatty acid beta-oxidation, was substantially increased. Also the transcription factor associated with oxidation, peroxisome proliferators-activated receptor-alpha, was enhanced in rat liver after 16 weeks of TO feeding. These data show important differences in the effect of TO-rich diet on hepatic lipogenesis and beta-oxidation depending on feeding duration. While, shorter term feeding a TO-rich diet results in progression of lipogenesis, the long-term TO feeding leads to inhibition of lipogenesis and intensification of fat oxidation.

### P10.39

#### Glyphosate based herbicides toxicity, a different approach

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Glyphosate is, probably, the most commercialized broad-spectrum herbicide worldwide. People may be exposed to its residues by agricultural practices or along the food chain. With respect to toxicity, harmful effects are described in several models, not only human. Among these effects are: cell cycle modifications, apoptosis and necrosis induction or alternations in gene transcription. We studied glyphosate toxicity in the human prostate cancer cell lines PC3 (androgen independent) and LNCap (androgen dependant). Results in cell proliferation by MTT salts metabolization, show a decrease in cell number up to a 60%, after 3 days of treatment with 50–100 µM glyphosate supplemented medium. Apoptosis induction analysis performed detecting Annexin-V by flow cytometry and valuating Procaspase-3 activation, shows an increase of apoptotic cells referred to control in both lines. Lactate Dehydrogenase (LDH) liberation assays indicate that there are significant levels of necrosis in LNCap line, but not in PC3. A primary study with propidium iodide by flow cytometry, apparently shows cell cycle alterations as well. All these effects, previously mentioned in other models, seem not to be enough to explain the dramatic decrease in cell viability we observed. That's why we studied an indicator of another cell death pathway: autophagy. Results on LC3 protein expression are highly significant, reaching a 200% signal referred to the control, both for 2 and 3 days of treatment, even before changes in apoptosis induction or LDH liberation are seen. The same occurs with acridine orange staining, which shows not only an increase of acid vesicles but also a redistribution of them. Commonly, all the effects are more intense in the androgen dependant cell line, what lead us to suspect that glyphosate has a differential effect between both models, resulting highly cytotoxic for LNCap and mainly Cytostatic in PC3.

### P10.40

#### Effect of quercetine and ascorbic acid on cytochrome P450 2E1 and oxidative stress in the liver of guinea pigs with type 2 diabetes mellitus

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The pathogenesis of diabetes mellitus, as a metabolic disease, is accompanied by the development of oxidative stress caused by high content of free radicals, a major source of which is cytochrome P450 2E1 (CYP2E1).

In the current study, we used a model of type 2 diabetes, which is based on the treatment of guinea pigs with protamine sulfate. The animals were divided into five groups of five animals each: the first group was intact guinea pigs; the 2–5 groups were intramuscularly injected twice a day with protamine sulfate (15 mg/kg) for 28 days. The second group of animals was used after this period for investigating the development of diabetes. The third group was kept at standard conditions for 14 days in order to control the remission of the disease. Animals of the 4th group were injected with a solution of bioflavonoid quercetin (CYP2E1 inhibitor) in the dose of 5 mg/kg. The 5th group was treated with both quercetin and vitamin C (20 mg/kg) for 14 days.

To evaluate the general condition of animals in all groups we determined the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), cholesterol and glucose levels in blood serum and the CYP2E1 level in the liver. The obtained data showed that after 6 weeks of the experiment animals of the third group have marked symptoms of diabetes such as 1,6-fold increase of glucose serum concentration. Hereby the slight increase in ALT activity and 1,45-fold increase in AST activity are evidences of liver injury. Furthermore the CYP2E1 protein level in the animals liver showed 1,5-fold increase. A significant decrease of these parameters took place in quercetin treated groups, but the greatest effect was observed in the group which was treated with both quercetin and vitamin C. These results indicate a pronounced hepatoprotective effect of quercetin which inhibits cytochrome P450 2E1 and thereby reduces the production of free radicals.

### P10.41

#### Effects of natural compounds in lipid profile and metabolism in hepatocytes

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Type 2 diabetes mellitus (T2DM) is associated with elevated triglycerides (TG) and LDL levels and decreased HDL levels, a pattern also recognized as dyslipidemia. Although the molecular mechanisms underlying diabetic dyslipidemia are not completely understood, this lipoprotein pattern is associated with T2DM and increased risk of cardiovascular diseases (CVD). Accumulation of fatty acids and lipid metabolites can inhibit insulin signaling pathway, leading to insulin resistant conditions. In a previous study, we reported the effect of *Salvia officinalis* tea in improving lipid profile in healthy female volunteers that constitute a risk to develop T2DM [1]. We also verified that the food constituents ursolic acid (UA) and luteolin-7-glucoside (L7G) had effects on plasma glucose and lipid profile improvement, whereas UA also

showed increased liver glycogen deposition and plasma HDL levels [2].

The present study aims to characterize the effects of natural compounds in lipid profile and metabolism of rat hepatocytes. Studies are underway to evaluate the *in vitro* effect of these compounds in lipid synthesis and/or degradation.

#### References:

1. Sá, CM, et al. *Int J Mol Sci* 2009; **10**: 3937–3950.
  2. Azevedo MF, et al. *Phytother Res* 2010, **24**: S220–S224.
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#### P10.42

### Mitochondrial diabetes in children from Southern Italy

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Maternally inherited diabetes and deafness (MIDD) is a rare form of diabetes characterized mainly by maternal inheritance of diabetes, hearing loss and macular dystrophy and is caused by mutations in mitochondrial DNA (mtDNA). The most frequent point mutation associated with MIDD is 3243A > G in the tRNA<sup>Leu</sup> (UUR) gene, that causes an altered ATP production and thereby impairs the glucose-induced insulin secretion.

The aim of this study was to search potentially diabetogenic mtDNA variants in a pediatric cohort from Southern Italy with suspected MIDD. We screened, by sequencing, the entire mtDNA, extracted from blood and buccal mucosa of MIDD patients (n = 11), of their mothers and of healthy control subjects (n = 80). We identified a total of 416 variants, among which 325 variants were detected only in controls, 58 variants were present both in controls and cases and 33 variants (4/33 novel) were present only in cases and their mothers. Among the variants detected only in patients, 22/33 were in the coding region (50% were synonymous and 50% caused an amino acid change). Only in one patient we found the 3243A > G variant. Because most patients (91%), and their mothers were mutated in complex I and/or IV of the mitochondrial respiratory chain, we measured by spectrophotometric assay, the enzymatic activities of these two complexes in lymphocytes of both patients and their mothers. We found that these enzymes were less active in mutated patients and their mothers than in the healthy control pool. Furthermore we observed a much lower prevalence of hearing loss (36% versus 75–98%) and macular dystrophy (54% versus 86%) in our mitochondrial diabetic children than reported in adults.

In conclusion our study support that the entire mtDNA should be studied in children with suspected MIDD and that the enzyme evaluation of mitochondrial respiratory chain complexes is a reliable method to assess the mitochondrial dysfunction associated with mitochondrial diabetes.

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#### P10.43

### Serotonin 5-HT<sub>2A</sub> receptors and NMDA glutamate receptors are involved in the molecular mechanisms of anti-diabetic effect of LVVYPW

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LVVYPW is a member of hemorphins family, an endogenous non-classical opioid peptides derived from hemoglobin and demonstrated a wide spread of biological activity by affecting different receptors function. Recently, we have revealed the blood glucose-lowering effect of LVVYPW in streptozotocin (STZ)-induced diabetic rats. Earlier it has been found that calcineurin is a key enzyme involved in the molecular mechanisms of hemorphins action in the brain and immune system. Because of functional interactions of calcineurin with serotonin 5-HT<sub>2A</sub> receptors and NMDA glutamate receptors, the aims of present work were to study the possible participation of mentioned receptors in anti-diabetic effect of LVVYPW. For that purpose STZ-induced diabetic rats (male, Wistar line, weighing 180–220 g) received the intraperitoneal (ip) pretreatment with either selective 5-HT<sub>2A</sub> receptor antagonist ketanserin (5 mg/kg) or NMDA receptor antagonist MK-801 (0.2 mg/kg) 30 minutes before ip administration of LVVYPW (1 mg/kg). It has been shown that pretreatment of diabetic rats with ketanserin completely abolished the blood glucose-lowering effect of hemorphin, while pretreatment of diabetic rats with MK-801 only partially inhibited (by 22.5%) blood glucose-lowering effect of hemorphin alone (by 36.5%) respectively. Thus, data obtained demonstrated that serotonin 5-HT<sub>2A</sub> receptors, as well as NMDA glutamate receptors are involved in molecular mechanisms of anti-diabetic effect of LVVYPW. These findings support the view that molecular mechanisms of anti-diabetic effect of LVVYPW are complex processes, which involve the integration of different receptors function with Ca<sup>2+</sup>/calmodulin/calcineurin/NFAT signaling pathway.

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#### P10.44

### Abscisic acid: a new human adipose tissue hormone?

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The plant hormone abscisic acid (ABA) behaves as an endogenous pro-inflammatory hormone in humans. ABA is released by activated inflammatory cells and stimulates several functional activities. ABA release is also induced in pancreatic beta cells after glucose administration and nanomolar ABA stimulates glucose-dependent and -independent insulin release.

The aim of this study was to investigate the role of ABA in the human adipose tissue (AT). Incubation of abdominal AT fragments from human biopsies with IL-8 or LPS for 24 hours induced a 1.5- to 8-fold increase of ABA content, respectively.