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PROTECTIVE ACTION OF NERVE GROWTH FACTOR (NGF) ON DEGENERATING RETINAL CELLS: NGF MEDIATES THE RESCUE EFFECT OF ADYPOCYTES?

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Nerve growth factor (NGF) is endogenous biological molecules produced and released but numerous neuronal and non-neuronal cells including adipocytes, we have recently reported[1]. We have also demonstrated that topical purified NGF administration on eyes exerts a protective action on degenerating retinal cells in glaucoma, maculopathy, diabetic retinopathy, and retinitis pigmentosa. Interestingly, there is indicating that intravitreal transplantation of adipose stem cells exerts protective action on retinal ganglion cells via secreted growth factors[2] and also in animal models of glaucoma[3] and in diabetic retinopathy[4]. Likewise, mesenchyme stem cells can be potential useful to rescue retinal degeneration[5]. Despite these evidences, mechanism through which adipocytes can actually secrete NGF *in situ* and lead to long-term benefits in the absence of undesired side effects is not full clear. The aim of the present presentation is to reveal findings regarding our data on the role of NGF on retinal cells and photoreceptors and discuss the hypothesis as to whether adipocytes present in the visual tissue might release NGF and be involved in the protective action on damaged retinal cells.

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ADIPOKINES IN METABOLIC SYNDROME

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The metabolic syndrome (MetSyn) is one of the pressing problems of civilization, which is caused by variety of clinical manifestations and lack of uniform criteria for diagnosis. It is a complex of metabolic disorders, which is based on obesity, dyslipidemia, insulin resistance, compensatory hyperinsulinemia, hypertention. Areas of active investigation focus on its molecular bases and potential pathogenic role of metabolic inflammation. A body of evidence suggests the presence of an overall, low-grade inflammation in obesity and MetSyn, with altered levels of several circulating factors such as an increase in the plasma levels of C-reactive protein, tumor necrosis factor-α, interleukin-6, transforming growth factor-beta and other biological markers of inflammation. We throw a light upon this question by studying the expression of some adipokenes - leptin, NGF and adiponectin in women with MetSyn and rats with MetSyn induced by high-fat-carbohydrate diet (HFCD).

Sixty premenopausal women with MetSyn and 60-age-matched clinically healthy women (age range 30-45 years) were included in the study. HFSD was applied in 20 male Wistar rats to induce obesity and MetSyn. Other 20 male rats which received standard rat chow were used as controls. Plasma leptin, NGF and adiponectin levels were measured by means of a solid-phase immunoenzyme assay (ELISA) performed according to the manufacturer's instructions (Human leptin ELISA, Human adiponectin (HEK) ELISA, Human NGF ELISA, BioVendor Laboratory, Medicin, Inc., Czech Republic). Immunohistochemical demonstration of leptin and NGF was carried out by the avidin-biotin peroxidase (ABC) method in subcutaneous white adipose tissue of the gluteal region.

The diagnosis metabolic syndrome was given after measuring the waist circumference, body mass index, glucose, insulin, total cholesterol and triglycerides plasma levels for each subject. The immunochemical results showed plasma levels of leptin and NGF of the women with MetSyn over-expressed ($P<0.01$). They correlated with body mass index, waist circumference, lipid profile and insulinemia in the subjects with MetSyn. Adiponectin levels were inversely associated with Met Syn. The application of HFSD induced MetSyn in the rats, which resulted in increased leptin and NGF plasma levels as compared with the control group ($P<0.05$). Unlike the women with MetSyn, in rat MetSyn the serum adiponectin levels were not significantly different than that of the controls. The immunohistochemical expressions in subcutaneous adipose tissue corresponded immunochemical data. The leptin and NGF reactions in Met Syn was stronger (+++) than that of the control group (+).

The application of HFSD in rats is a good experimental model for inducement of metabolic syndrome. It successfully imitates the pathology in humans. Our results suggest the possible role of the adipokines: leptin, NGF and adiponectin as inflammatory molecules in the pathogenesis of metabolic syndrome.

HYDROGEN SULFIDE MEDIATES ENHANCED LIPOLYSIS IN VISCERAL ADIPOSE TISSUE OF OBESE RATS

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Lipolysis is the principal metabolic function of the adipose tissue. Obese animals and humans are characterized by enhanced baseline lipolysis especially in visceral adipose tissue. Excessive release of non-esterified fatty acids contributes to insulin resistance and lipotoxicity in remote tissues. We examined the role of H_2S, the endogenous "gasotransmitter", in the regulation of AT lipolysis.

Microdialysis probe was inserted into the mesenteric fat depot of anesthetized rats and was perfused with Ringer's solution at the rate of 1 μl/min. Fractions were collected in 10-min intervals and the rate of lipolysis was assessed by measuring glycerol concentration in the dialysate.

H_2S donor, Na_2S, increased glycerol release in a concentration-dependent manner. In addition, Na_2S increased cAMP production and its lipolytic effect was abolished by protein kinase A inhibitor, KT5720. Glycerol release was significantly higher in mesenteric AT of rats made obese by feeding highly palatable diet for 1 month. H_2S concentration in the dialysate was by 168% higher in obese animals. Propargylglycine, the inhibitor of H_2S-synthesizing enzyme, cystathionine γ-laye (CSE), reduced lipolysis in lean and obese rats and abolished the difference in glycerol release between these two groups. Next, we examined why H_2S is higher in AT of obese rats. H_2S is synthesized from L-cysteine by CSE and is enzymatically oxidized in mitochondria. CSE expression and activity were similar in lean and obese rats. In addition, H_2S production by adipose tissue explants measured in the presence of stigmatellin, the inhibitor of mitochondrial H_2S oxidation, did not differ between groups. However, mitochondrial H_2S oxidation, measured as the difference in H_2S production in the presence and in the absence of stigmatellin, was markedly reduced in obese rats. Finally, we looked at several factors which determine the rate of mitochondrial H_2S oxidation. First, mitochondria density, measured as the expression of cytochrome C and citrate synthase activity, was similar in lean and obese rats. Second, the activity of sulfide:quinone oxidoreductase (SQR), the rate-limiting enzyme in mitochondrial H_2S oxidation, as well as the concentration of ubiquinone (the SQR cofactor) did not differ as well. However, oxygen tension was 3-fold lower in mesenteric AT of obese rats. Finally, H_2S production by AT explants incubated at 5% O_2 was higher in obese than in lean rats, however, increasing O_2 concentration to 21% markedly reduced H_2S production and abolished the difference between both groups.

(i) Exo- and endogenous H_2S stimulates lipolysis in visceral adipose tissue in cAMP-protein kinase A dependent manner, (ii) H_2S production in visceral adipose tissue increases in obesity, and H_2S drives enhanced lipolysis rate, (iii) enhanced H_2S production in AT of obese rats does not result from increased synthesis but rather from reduced mitochondrial oxidation due to adipose tissue hypoxia. The results indicate that adipose tissue hypoxia, by enhancing net H_2S production, plays a key role in hyperlipolytic state associated with obesity and thus may contribute to complications such as insulin resistance and lipotoxicity. Inhibiting H_2S system may be a novel treatment strategy to combat detrimental metabolic consequences associated with the metabolic syndrome.

CHARACTERISTICS OF THE ALCOHOLIC PATIENTS WITH HYPOGLYCAEMIA

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Several studies have shown that alcohol abuse can lead to hypoglycaemia. Although it is not known to what extent, this correlation is well explained in terms of pathophysiology. For this purpose we observed a population of alcoholic patients suffering from hypoglycaemia. We conducted a retrospective study in the Emergency Department of Policlinico Umberto I in Rome, analyzing all the entrances and all the patients coming with hypoglycaemia from January 2012 to December 2013. We assessed the patients with blood glucose levels less than 70 mg/dl. In so doing, we recruited 441 patients identified as the target population, and we also selected a group of 52 patients showing hypoglycaemia associated with acute alcohol intoxication or chronic alcoholism. Comparing the group of alcoholics and the main population, it turned out that there was a predominance of male sex, and a lower average age among alcoholics with hypoglycaemia with regard to the entire hypoglycaemic population Moreover in the group of alcoholics there was a higher incidence of consciousness alterations, whereas average blood glucose levels and the presence of hepatic impairment show no difference between the two groups.

OBESITY RELATED ALTERATIONS IN DRUG DISPOSITION AND PHARMACOKINETICS: EMERGING CLINICAL IMPLICATIONS IN OBESE PATIENTS

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Obesity is characterized by the abnormal or excessive accumulation of body fat with a body mass index (BMI) ≥ 30.0 kg/m^2, whereas the BMI of non-obese individuals ranges from 18.5-24.9 kg/m^2. This non-communicable but chronic disorder contributes heavily to major risk factors involved in the occurrence of type 2 diabetes, osteoarthritis, cancer, and cardiovascular and neuropsychiatric diseases, all of which can potentially lead to severe morbidity and mortality. Extreme obesity (BMI 35-40 kg/m^2) also causes stress on the respiratory system, decreasing the individual's functional residual capacity. Obesity-induced cardio-respiratory stress interferes with the cardiovascular system by demanding an increased circulating blood volume, and consequently subjecting the left ventricle to both pressure and volume overload. These factors subsequently change drug's pharmacokinetics (PK) and disposition in obese patients. Many co-morbid conditions associated with obesity require polypharmaceutical interventions. Administration of different types of drugs may not only cause adverse drug-drug interactions but may also produce changes in PK and pharmacodynamics (PD) of drugs, especially lipid soluble agents. Differences in volume of distribution or clearance that result from excess adipose tissue can lead to improper dosing, often leaving patients mistreated for their ailments. Obesity related changes in metabolism, distribution, PK and PD parameters of lipid soluble drugs may profoundly alter in obese patients, thereby requiring drug dose adjustment. Adipose tissue contains CYP17 and CYP19 and is capable of producing sex steroid hormones like the testes and ovary (1). The sex steroid hormones originating from adipose tissue may cause induction/inhibition of major drug metabolizing enzymes in the liver ($CYP_{450}3A4$, $CYP_{450}2D6$), and consequently modify drug metabolism/disposition in obese subjects. It has been reported that xenobiotic-metabolizing CYP_{450s} are also expressed in the adipose tissue, especially CYP1A1 and CYP1B1 that can bioactivate carcinogenic polycyclic aromatic hydrocarbons and exogenous estrogens (2). In view of all these factors, inclusion of obese men and women in clinical trials is needed for better understanding the distribution, biodegradation, PK and PD profiles of old and new drugs in obese patients.

With the increasing population of overweight and obese people in today's obesogenic society, it is vital that dosing regimens for antimicrobials, anticancer agents, anesthetics, analgesics, oral contraceptives, and antipsychotics are also formulated for obese subjects and not only for lean individuals. It is no longer sufficient to base drug doses on size metrics such as body weight alone. This communication will provide an updated overview about the metabolic disposition, PK and PD changes of a wide spectrum of drugs in obese and non-obese patients.

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RESISTIN/ADIPONECTIN/FGF21 INTERACTIONS IN T2DM RODENT MODELS

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Obesity and type2 diabetes share several features such as insulin resistance and energy homeostasis deregulation driven by changes of adipose tissue secreted hormones. Indeed, adiponectin, an insulin-sensitizing hormone, and resistin, known to promote insulin resistance, are potential links between obesity and type2 diabetes. In addition, Fibroblast growth factor 21 (FGF21), predominantly produced by the liver, has similar effects as adiponectin in regulating glucose/lipid metabolism and insulin sensitivity. However, the interplay between adiponectin, FGF21 and resistin signaling pathways in the onset of insulin resistance is unknown. In the present study, we (1) investigated whether central resistin promotes insulin resistance through the impairment of adiponectin signalling and by promoting FGF21 resistance and (2) whether we can block resistin action using resistin mutant acting as resistin antagonist.

Chronic ICV resistin infusion down regulated both hypothalamic and hepatic APPL1, a key protein in adiponectin signalling, associated with decreased Akt/APPL1 interaction and an increased Akt association with its endogenous inhibitor TRB3. Resistin treatment also reduced the expression of adiponectin receptors in hypothalamus, liver, muscle and adipose tissue. Furthermore central resistin acting through TLR4 impaired insulin sensitivity consequently to the downregulation of FGF21 and its receptor components in the hypothalamus and peripheral tissues promoting FGF21 resistance. We also showed that resistin effects are abolished in TLR4 knock-out mice and in cells expressing TLR4 siRNAs.

To block resistin action in mice fed high fat diet (HFD) that are prone to obesity and inflammation, and in attempt to reverse these metabolic disorders we have developed and purified to homogeneity recombinant human resistin mutant that acts as resistin antagonist (RA). We tested the efficacy of RA in human neuroblastoma cell line SH-SY5Y and in mouse hypothalamic cell line mHypo 280. First, we showed that resistin- induced the phosphorylation of Akt could be blocked completely and in lower doses gradually abolished. Once, the efficacy of RA in vitro was demonstrated, we attempted to reverse the HFD-dependent insulin resistance by RA treatment in vivo. For this purpose, we have fed male C57BL/6 mice with HFD for 6 weeks and then mice received for 14 days daily injection of RA (0.3 mg/day/mice), while a control group was that received chow diet was treated similarly. We show that RA led to a significant decrease in body weight of HFD mice mainly due to loss of visceral fat and restored glucose tolerance and insulin-responsiveness as evidenced respectively by glucose tolerance test and by insulin tolerance test. In conclusion, we demonstrated that the blockade of resistin action reduced body weight gain, visceral fat content and restored insulin responsiveness of mice fed HFD.

In summary, our study reveals novel mechanism explaining the onset of insulin resistance orchestrated by central resistin/TLR4 pathway that impairs adiponectin signaling and promotes FGF21 resistance.

ADIPOBIOLOGY: A RESEARCH FIELD MARKED BY FIVE PARADIGM SHIFTS

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In this candlelight lecture we will start with a brief historical survey. Admittedly, the accumulation of adipose tissue (AT) was linked to obesity. This was known to Hippocrates, who stated that "sudden death is more common in those who are naturally fat than in the lean". Thomas Short's book, *A Discourse Concerning the Causes and Effects of Corpulency* (1727), was the first English language monograph on obesity. The adipocyte, firstly dubbed "fat vesicle", was recognized as a specific cell type of AT by Hassall (1849). Recently, AT has taken a center stage in many diverse diseases beyond obesity. Based on this intellectual growth process a new field of research, adipobiology, was conceptualized (1). Since the discovery of leptin (1994), AT has undergone five major paradigm shifts (in sense of Thomas Kuhn). The first paradigm shift: from merely a fat storage, AT is now known as a major endocrine and paracrine organ of the human body, producing more than 200 signaling proteins (adipokines) (2). The second paradigm shift: external *versus* internal adipose depot that spotlights our knowledge about TOFI (thin outside, fat inside) and related phenotypes (3). The third paradigm shift: white *versus* brown adipocytes, recovering the significance of brown adipobiology (4). The fourth paradigm shift: a link between AT and the human exposome, that is, adipotoxicology (5). The fifth paradigm shift: the involvement of adipokines in the pathogenesis of psychiatric (6) and neurodegenerative diseases, including adipose-Alzheimer (7).

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UNRAVELING THE OXIDATIVE POTENTIAL OF EPICARDIAL FAT IN HUMANS

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Epicardial adipose tissue is a unique fat depot around the heart that shares a close anatomic proximity and vascular supply with the myocardium and coronary arteries. Its accumulation around the heart, measured using various imaging modalities, has been associated with the onset and progression of coronary artery disease in humans. We, and others, have reported that human epicardial adipose tissue expresses uncoupling protein 1 (UCP1), a marker of brown adipocytes, at both mRNA and protein levels. Presence of UCP1 was also associated with a higher capacity for oxidizing fatty acids in epicardial fat relative to subcutaneous fat in our cohort. In addition, expression levels of most genes involved in thermogenesis including UCP1 exhibited significant positive correlations with HDL-cholesterol while simultaneously exhibiting negative associations with circulating triglyceride levels, indicating that the thermogenic capacity of epicardial fat shares an association with the systemic metabolism in humans. Since then, we have expanded our work to investigate whether human epicardial fat represents a beige fat depot *i.e.* a fat depot that carries UCP1 positive adipocytes, and is capable of upregulating leak respiration upon stimulation. Using direct tissue sampling and *in-vitro* differentiated primary adipocytes derived from patients undergoing heart surgeries, we have observed that human epicardial fat carries the molecular signatures of a beige fat depot and can be stimulated to upregulate its thermogenic machinery. Our preliminary observations of the presence of natriuretic peptide receptors and beta-klotho in epicardial fat further suggest that it could serve as the thermogenic target site for circulating peptides such as natriuretic peptides and FGF21. The relevant question of whether the thermogenic properties of epicardial fat can be targeted to manage its mass in humans remains open for exploration.

EPIGENETICS OF OBESITY

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The importance of epigenetics for health and disease is widely recognized. Epigenetic factors play as important role in determining phenotype as genetic factors are and can also be heritably transmitted to successive generations. Technological advances in epigenome profiling caused increase of research in the field of epigenetics in obesity. Obesity and related cardiometabolic diseases such as atherosclerosis, hypertension, type 2 diabetes and metabolic syndrome are associated with epigenetic changes. There is also evidence that early life environmental exposures (including *in utero*) can lead to stable alterations in the epigenome leading to increased risk of obesity later in life. Risk factors other than nutritional, like inflammation, hyperglycemia and oxidative stress also seem to be linked to these epigenetic changes. Epigenetic research currently has several major objectives: (i) to identify epigenetic biomarkers predictive for future risk of development of obesity, (ii) to identify environmental factors, associated with obesity, and (iii) to find therapeutic, nutritional and/or pharmacologic designs that could modify the epigenome. The first feasible epigenetic markers detectable at birth have been identified and predicting obesity at this age is a prerequisite for targeted prevention strategies. It has been shown that changing exposure *in utero* and changes in adult lifestyle can modify the adverse epigenetic profiles. Active ingredients able to modify the epigenome and their doses and also optimal period of life for interventions need to be identified. This new layer of our understanding of obesity holds promise for more elegant and effective treatment.

OLIVE POLYPHENOLS' ROLE IN REGULATING METABOTROPHINS AS NGF AND BDNF

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Polyphenols are probably the most known and investigated molecules of nutrition interest and they are micronutrients present in abundance in our diet. Some of the most important food sources of polyphenols are olives and olive oil. A growing body of evidence from animal models and clinical studies indicates that polyphenols have neuroprotective effects in various pathological states of the nervous system through the control of oxidative stress, inflammation, apoptosis, mitochondrial dysfunction. Also at peripheral level, they act as antioxidant, defending tissues against oxidative damage and scavenging free radicals. Moreover they prevent cardiovascular disease by regulating the plasma levels of oxided LDL, decrease postprandial triacylglycerols, increase HDL and have also anti-thrombotic effects. Additionally, a number of recent experimental and clinical data suggest that olive polyphenols are able to alter the expression of the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) primarily known as biological mediators stimulating neuron growth, survival and differentiation (1-4) and recently studied also as metabotrophins, acting on glucose, energy, pancreatic beta cells and cardiovascular homeostasis (5). In this context, better understanding the effects of polyphenols on these neuro-metabotrophic molecules certainly could generate interest for drug discovery and also for the potential dietary prevention of several cardiometabolic and neurodegenerative diseases.

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OSTEOCALCIN IN A RAT MODEL OF METABOLIC SYNDROME

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Recently, a research group led by Gerard Karsenty at Columbia University in New York, USA has demonstrated that bone is an endocrine organ that secretes a multifunctional hormone, osteocalcin (OC), and proposed a regulatory circuitry involving bone, pancreas, adipose tissue and brain in maintaining energy homeostasis in mice (1). The principal hormonal role in this loop has been ascribed to OC. This osteoblast-derived (and vitamin K dependent) protein has been shown to induce insulin secretion and beta cell proliferation and to improve insulin sensitivity. It also increases adiponectin and decreases leptin release from the adipocytes. Osteocalcin has been postulated to be metabolically active in mice in its un(der)carboxylated form. This groundbreaking research was immediately reflected by clinical studies that were undertaken to test the hypothesis in humans. Results are so far inconsistent and equivocal. Although in general many papers have reported various associations between OC and parameters of energy metabolism, serious discrepancies still exist in the literature as to which form of OC, the carboxylated or the un(der)carboxylated one, is responsible for the metabolic activity in man.

Whether OC serves a similar metabolic role in the rat is currently unknown. In this pilot study we looked at OC in rats fed Western type hyper-caloric diet to develop a condition similar to metabolic syndrome (MS) in humans.

Two groups of rats were used. The control group received regular rat chow and plain water throughout the experiment. The experimental group was fed high fat (17 % lard) and high (17 %) fructose (HFHF) diet and had 10% fructose in their drinking water. The duration of the experiment was 10 weeks. Insulin tolerance test (ITT) was performed at the end of the experiment; serum lipids were measured; serum insulin, leptin and both forms of OC – carboxylated and udercarboxylated, were determined by ELISA kits. HOMA-IR was calculated.

Body weight of the animals did not differ after 10 weeks of dieting, but the diet manipulated rats had higher caloric intake. The metabolic syndrome induced by HFHF feeding was verified by increased visceral adipose tissue, elevated serum triglycerides (TGs) and blood glucose, positive ITT at the 90th minute, higher insulin and leptin levels. Undercarboxylated osteocalcin (ucOC) in the serum was reduced in the HFHF rats, whereas carboxylated osteocalcin (cOC) was slightly and insignificantly increased. The ratio ucOC/cOC was also lower in the experimental group. Undercarboxylated osteocalcin and ucOC/cOC were inversely associated with blood glucose in the HFHF group. No associations were found between ucOC and insulin, leptin, visceral adipose tissue or TGs. Carboxylated OC showed no correlation with any of the metabolic parameters examined.

Our preliminary results support partly the hypothesis that ucOC might be implicated in energy regulation also in the rat. In this animal species the undercarboxylated form of OC is probably the metabolically active one. Nevertheless, compared to the reported effects in mice, OC in rats seems much less active. More evidence is necessary to confirm the hormonal role of OC in the rat.

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ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA: ANOTHER EXAMPLE OF ADIPOSE TISSUE RELATED DISEASES – INVOLVEMENT OF NEUROTROPHINS

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Arrhythmogenic right ventricular dysplasia (ARVD), also known as fatty degeneration of right heart, is a heritable disorder characterized by progressive degeneration and fibro-fatty replacement of right ventricular myocardium, causing electrical instability of the right ventricular myocardium, ventricular tachyarrhythmia and sudden death at a young age (1). Several lines of evidence suggest that impairment of cardiomyocyte adhesions (desmosomal proteins) may be the underlying pathogenic mechanism *via* accelerating apoptosis of these cells (2), but ARVD pathogenesis is still unclear (3). Since (i) adipose tissue replacement of cardiomyocytes is the most essential histological finding in ARVD, and (ii) nerve growth factor (NGF) exerts an arrhythmogenic effect related to sudden cardiac death (4), the aim of the present study was to analyze immunohistochemically ARVD-related adipocytes with special attention to the expression of NGF and related neurotrophins, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) and their respective TrkA, TrkB and TrkC receptors. Eight cases with ARVD were autopsy proven. The present results show that the intramyocardial adipocytes in ARVD expressed these neurotrophins and their receptors, thus suggesting that they may play a substantial part in life-threatening myocardial electrical instability.

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PTEN, HSP27 AND NFkB IN ATHEROSCLEROSIS - AN IMMUNOHISTOCHEMICAL STUDY

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Phosphatase and Tensin homologue deleted on chromosome10 (PTEN) is a multifunctional tumour suppressor gene.It mediates several cell functions such as migration,proliferation and apoptosis.These are mediated by antagonism of the phosphatidyl inositol 3 kinase (p13k) mediated signalling pathway. In atherosclerosis inflammatory cytokines and chemokines play important roles in cell proliferation and migration. Overexpression of PTEN reduced neointima formation in association with inhibition of macrophage invasion,cell proliferation and expression of proinflammatory cytokines in addition to suppression of cell proliferation,migration and increased apoptosis.

Heat shock proteins(HSPs) are molecular chaperones that protect against stress stimuli inducing heat shock,oxidised LDL, mechanical stress,oxidants and cytokine stimulation. HSP27 belongs to the small HSPs family.It inhibits F-actin polymerisation, protects against apoptosis,and it presents oxidised proteins to the proteosome degradation machinery. HSP27 expression is increased in the normal appearing vessel adjacent to an athersclerotic plaque.Factors which enhance the production of HSPs may necessarily lead to reduced proliferation of smooth muscle cells and control the plaque size. Nuclear factor kappa B(NFkB) is a transcription factor which mediates the production of inflammatory cytokines by vascular cells,monocyte adhesion and proliferation,migration and proliferation of smooth muscle cells.Its activation leads to an increase in expression of many cytokines,adhesion molecules and enzymes involved in inflammation and proliferation.Activation of NFkB is an early event in atherosclerosis.It is present in fibrotic thickened intima media and atheromatous areas including smooth muscle cells, macrophages and endothelial cells whereas it is totally absent in nonatheromatous areas of the vessel.Several cytokines such as TNFalpha and Interleukin-1 are able to activate NFkB in vitro.Activated NFkB may also modulate the chemotactic substance produced by the endothelial cells,such asMCP-1(monocyte chemotactic protein-1) that attracts leucocytes to the lesion.Activation of signalling pathways by NFkB mediates cell survival mechanisms and contrarily it could also modulate pathways leading to cell degeneration,aging, disease and death.

Immunohistochemistry of the atherosclerotic lesions.The study of the aterosclerotic lesions in different stages and comparing it to the normal vessel with the help of immunohistochemical staining using the appropriate antihuman antibodies against the above proteins would help us to locate the proteins and predict the outcome of the disease .The presence of PTEN and HSP 27 are good pointers to the absence of the disease whereas presence of NFkB would be an indicator of atherosclerotic vessel disease. Besides immunohistochemistry could also prove to be a good tool for the assured presence of vessel disease and in some cases point out the presence of an early plaque. This could be used as a useful adjunct to the other diagnostic procedures in the pathology laboratory.

NUCLEAR RECEPTORS RXR AND LXR IN BRAIN – MORE THAN LIPID METABOLISM

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The research on the biology and function of the family of transcription factors called nuclear receptors (NRs) has influenced and changed dramatically our understanding of basic regulatory and metabolic pathways. The therapeutic implications of this knowledge are enormous. Most of those nuclear receptors can not do their job alone, however, and their regulatory function is in fact executed while bound to DNA response elements as permissive heterodimers with Retinoid X Receptors (RXRs). Some of the most prominent examples of the so called Class II NRs that require RXR for heterodimerazition are PPAR, LXR and TR. In the last 3-4 years a significant attention has been directed towards RXR for several reasons: **A)** in addition to working as heterodimers with other NRs, RXR form homodimers; **B)** RXR/RXR homodimers may have their own responsive genes, or once activated may activate genes, traditionally considered LXR/RXR, PPAR/RXR or even targets genes of the non-permissive RAR/RXR heterodimers; **C)** there are synthetic and receptor type specific RXR ligands - one of them Bexarotene (Targretin) that have been FDA approved and for many years now in clinical use. Importantly it has been shown repeatedly, that ligand-activated RXR ameliorate memory deficits in Alzheimer's disease mouse models including mice expressing human APOE isoforms. There has been however, incomplete understanding about molecular mechanisms underlying the effect of those ligands in the brain, moreover, the cognitive impairment in different mouse models does not correlate to Aβ deposition in brain parenchima or vasculature. I will present the very recent research in our laboratory with the goal to gain further insight into molecular mechanisms whereby ligand-activated RXR can affect or restore cognitive functions. Our overall unbiased approach is based on revealing genome-wide changes in RXR cistrome (ChIP-seq) and gene expression profile (RNA-seq) in response to bexarotene in the cortex of APOE4 mice. The results of Next Generation Sequencing have been accordingly validated and further used to examine Functional Gene Ontology categories enriched in both data sets and they show bexarotene-liganded RXR affect signaling pathways associated with neurogenesis and neuron projection development. Further validation assays in mouse embryonic stem cells (ES), primary neurons and APOE3 and APOE4 mice treated with bexarotene confirm the results. Altogether the results of our studies on the role of bexarotene-activated RXRs in brain provide further evidence that they promote genetic programs involved in neurogenesis and development of neuronal projections with significance for improvement of cognitive deficits in an array of neurological, psychiatric and neurodegenerative disorders.

ADIPOCYTES AS BIOLOGICAL PROMOTERS OF WEIGHT REGAIN AFTER WEIGHT LOSS

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Losing weight is an appropriate way for obese subjects to significantly improve their health status and reduce morbidity risk. A popular method is taking a low calorie diet, which is often successful. Unfortunately, up to 80% of people do not succeed in keeping their weight at the reduced level. This weight regain is usually attributed to psychosocial influences. However, we propose that structural and metabolic changes in adipocytes during weight loss increase the risk for weight regain. One such change is that during, or shortly after weight loss, adipocytes increase their capacity for uptake of fat. During weight loss, adipocytes lose fat and shrink. We suppose that under a negative energy balance, the extracellular matrix (ECM) cannot match this reduction in volume. As a consequence, traction occurs between the ECM and the cell, which generates cellular stress. One way to reduce this adipocyte stress is by accumulating fat and allowing the cells to return to their original volume. This can be achieved by adjusting the adipokine levels. Figure 1 provides a schematic overview of our hypothesis. Experiments have been done to provide evidence for this hypothesis. First, we have investigated if indeed cellular stress in adipocytes rises during weight loss, both *in vivo* and *in vitro*. Second, we have searched for genetic association between the risk for weight regain and genes coding for ECM-components.

Adipose tissue biopsies were taken from subjects that lost 10% body weight in 5 weeks on a very-low caloric diet. Weight was measured again 10 months after the weight loss phase. Levels of stress proteins were determined by Western blotting before and after weight loss. In parallel, SGBS cells were glucose-restricted for 4 days and levels of the same stress proteins were determined. For the genetic association study, SNPs in and around 129 genes coding for ECM proteins in adipocytes were analysed for association with weight regain/maintenance after weight loss.

After weight loss, the level of some of the stress proteins was significantly higher in the group of subjects that regained weight than in those who maintained the reduced weight. This included beta-actin and HSP27, which are related to so-called stress fibers.

Genetic association was detected between weight regain and five ECM-genes with differences between males and females.

Our findings are in keeping with a model in which adipocyte cellular stress, accumulating during weight loss, is related to the risk for weight regain. Moreover, our genetic observations are in line with a role for the ECM in the risk for weight regain.

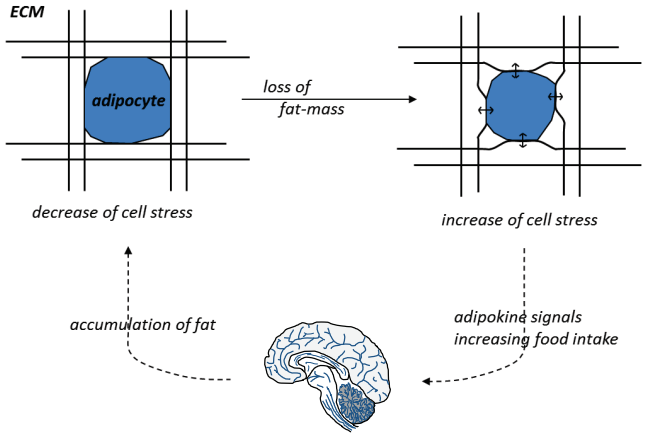

Figure 1. *A model for weight regain via adipocyte cell stress.*

ADIPONECTIN – A POSSIBLE LINK BETWEEN AGEING, METABOLIC STRESS AND OXIDATIVE STRESS?

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Ageing is associated with numerous hormonal changes affecting the energy metabolism and sensitivity to insulin. Evidence suggests that a clustering of sources of oxidative stress exists in obesity: hyperglycemia, hyperleptinemia, increased tissue lipid levels, inadequate antioxidant defenses, increased rates of free radical formation, enzymatic sources within the endothelium, and chronic inflammation. Adiponectin is a highly abundant insulin sensitizing, anti-inflammatory, and anti-atherogenic protein hormone produced exclusively by adipocytes. The aim of the present study is to explore the potential link between circulating levels of adiponectin, general metabolic profile, endothelial function, and systemic glycoxidative/lipoxidative stress in elderly subjects with type 2 diabetes and hyperlipidemia.

53 subjects (11 men and 42 women, aged 60+17 years), hospitalized at NIGG „Ana Aslan", were divided in two study-groups: a control group (n=23), including healthy patients with normal values of biochemical and hematological parameters; a group of patients with impaired fasting glucose (IFG) or type 2 diabetes mellitus (DM2) and hyperlipidemia (n=30). Serum adiponectin levels were evaluated together with a biochemical marker of endothelial dysfunction - nitric oxide metabolic end products NOx [NO2- + NO3-], and serum oxidative stress parameters: Advanced Oxydation and Glycation Protein Products (AOPPs and AGEs), total oxidative and antioxidative capacity (TOC and TAC), low density lipoprotein susceptibility to lipid peroxydation (LDLox).

Our results clearly pointed out significant lower levels of adiponectin in elderly hyperglycemic subjects compared with healthy age-matched group, concomitantly with significantly higher levels of oxidative stress and endothelial dysfunction markers. As well, patients with chronic hyperglycemia had significantly higher NOx values compared with the control group. Lipid and lipoprotein parameters and not glycemia, were the main metabolic determinants of adiponectin. Serum levels of adiponectin positively and significantly correlated with HDL-cholesterol as well as with total cholesterol, both in hyperglycemic subjects and in all study population. Stratified analysis according to serum adiponectin levels, in all study subjects (n=53) showed significantly higher values of AOPP, LDLox and NOx, for subjects with lower adiponectin levels (< 14 ng/ml).

The marked increases in oxidative stress markers in impaired glucose metabolism elderly subjects could be due to cumulative effects of adiponectin secretion deficiency and HDL reduced levels, which both contribute to the decrease in antioxidant activity, favoring oxidative processes. Adiponectin in combination with NOx, LDLox and AOPP appear to be important biomarkers for evaluating the association between metabolic imbalance and systemic oxidative stress in elderly patients.

FROM ADIPOSE TISSUE TO THE BONE – OUR EXPERIMENTAL STUDIES

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Adipose tissue, in addition to other cellular components, is also a rich source of adult stem cells, thus being an important candidate for participation in the regenerative medicine and tissue engineering. In our experimental studies of bone regeneration and bone tissue engineering, we used adipose tissue in a number of ways and in a several experimental models.

With the components of adipose tissue, we used mineral bone matrix as a carrier for the cells or as a scaffold for new bone tissue. We have used blood components such as platelet-rich plasma as a supplement to such constructs in some of the studies[1,2]. We have also made preliminary experiments with 3D printed scaffolds of bone tissue. Adipose tissue was used in various forms such as chopped whole tissue, freshly isolated stromal vascular fraction, expanded adipose-derived stem cells (ADSCs) as well as ADSCs *in vitro* osteo-induced[1] and induced into endothelial cells[2]. The potential of the adipose tissue in the bone formation were studied in orthopic and ectopic osteogenic models in rabbits, mice and rats, as well as in cell cultures *in vitro*. Assessment of osteogenic potential was performed using numerous methods including histological staining, morphometry, immunohistochemistry, radiographic and analytical methods, analysis of specific gene expression, various *in vitro* methods and others[1,2]. All experiments have shown that adipose tissue expressed osteogenic potential, so that it can participate in the formation of new bone, inducing, enhancing and favoring osteogenic process. Components of adipose tissue and forms in which they were applied have shown osteogenic potential in different degree and expressed it in different ways[1,2].

The obtained data may be applicative in regenerative medicine of skeletal system because they may help in the choice of a way of using the adipose tissue in accordance with the goal of treatment.

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PHENOTYPE MODULATION IN BROWN ADIPOSE TISSUE: BEIGE, BRITE AND BRUSCLE ADIPOCYTES

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This lecture aims to cover some aspects of the recent progress in our understanding of the anatomical, developmental, potential differences in responsiveness and functional characteristics of brown, beige and bruscle adipocytes, with a special emphasis on adult human brown adipose tissue (BAT). The attitude that white adipose tissue (WAT) has merely lipid storage and supporting functions has been completely changed in the last decades. Now, WAT is identified as a dynamic endocrine and paracrine organ secreting pro- and anti-inflammatory signaling proteins (adipokines), the adipokine imbalance leading to cardiometabolic diseases. Contrary, BAT may be considered as a health-promoting organ suppressing the development of these diseases through its thermogenic function (1). Brown adipocytes burn glucose and lipids to maintain thermal homeostasis and dissipate energy to produce heat through nonshivering thermogenesis, via mitochondrial uncoupling proteins. Thermogenic adipocytes are classified as classical brown adipocytes and beige (also referred to as brite) adipocytes with distinct developmental and anatomical features. Brown adipose tissue was considered to be present and active in prematures, neonates and young children generating heat and helping thermoregulation; it may transform into WAT, known as brown-to-white transdifferentiation (2). The main BAT depots are disseminated throughout the human body (around the aorta, common carotid artery, brachiocephalic artery, around epicardial coronary artery, in anterior mediastinum, supraclavicular fossa, axilla, thoracic paravertebral loci, between neck muscles) (3). Some new data suggest that brown adipocytes might be interspersed in WAT of rodents and humans to form 'beige cells' or 'brite fat cells'(brown in white) as a result of chronic cold exposure (4-6).
In effect, these new data might be implicated in BAT-focused strategies in the prevention and therapy of obesity and related cardiometabolic diseases.

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OBESITY AND CANCER, ROLE OF ADIPOKINES IN CANCER

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Obesity is associated with cancer development and progression. Meta-analyses showed that the risk for several types of cancer increased with 6-59% with every increment of 5 kg/m^2 in BMI above normal levels. Vice versa, weight loss reduces the risk for cancer. Epidemiological studies further indicated that, next to cancer development, cancer progression is worsened in obese subjects. Cancer progression is particularly driven by metastasis. While the association between obesity and cancer is increasingly evident the molecular mechanisms involved in obesity-mediated neoplasia and/or metastasis are still elusive. One hypothesis states that fat tissue in close proximity with tumors forms a permissive microenvironment. This is explained by cancer-mediated lipolysis, which gradually changes adipocytes into cancer-associated fibroblasts (CAF's). Subsequently, the secretome of these CAF's is altered with increased expression of pro-inflammatory adipo(cyto)kines. An alternative hypothesis is that obesity-induced alterations in circulating adipokines levels may result in elevated cancer risk. Decreased adiponectin and increased leptin levels directly affect cancer development and progression in *in vitro* and *in vivo* models. Despite the established relation between weight gain/loss and cancer, the potential metastasis-inducing effect of the human adipocyte secretome is barely investigated.

Hallmarks of metastasis are invasion and migration of primary tumor cells to distal sites to form secondary tumors. The migration potential of human adipocyte secretomes on human HT-29 colorectal adenocarcinoma cells was investigated with a coculture system (see Figure 1). HT-29 cells were incubated for 48 hrs with conditioned medium derived from SGBS preadipocytes, SGBS adipocytes and SGBS adipocytes starved for 4d and 20d. Migrated cells were detected with a crystal violet assay. The SGBS secretomes were analyzed by liquid chromatography tandem mass spectrometry (LC-MSMS) to identify the secreted proteins.

Compared to the preadipocyte secretome, the adipocyte secretome significantly induced HT-29 cell migration. With the secretomes of starved adipocytes migration of HT-29 cells was comparable with the migration observed with the preadipocyte secretome. Proteome analysis of the secretomes revealed that the SGBS adipocyte secretome contains several proteins with functions related to cellular proliferation and migration.

Secretomes of human subcutaneous adipocytes induce migration of human colorectal adenocarcinoma cells. This effect can be reduced by starvation. These results indicate that obesity may induce colorectal cancer metastasis.

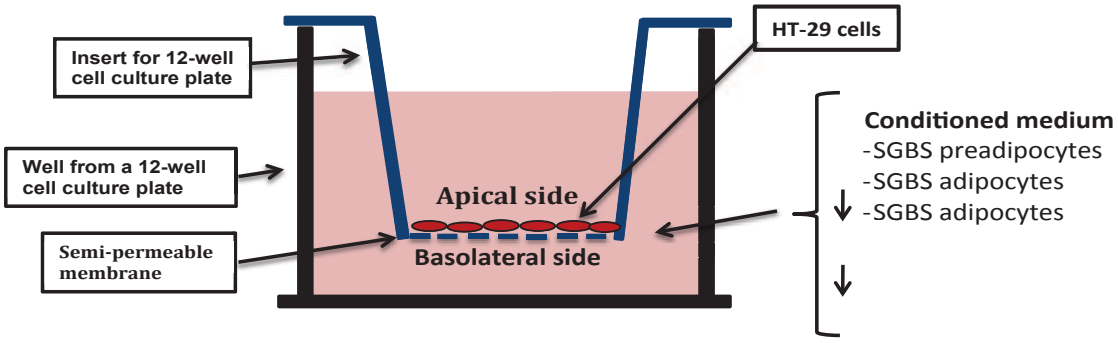

Figure 1. *Co-culture set-up used to investigate the migratory potential of the SGBS adipocyte secretome on human HT-29 colorectal adenocarcinoma cells.*

IMAGING HEART-ASSOCIATED ADIPOSE TISSUE BY LATEST GENERATION DUAL-SOURCE-COMPUTED TOMOGRAPHY CT FORCE: PRELIMINARY DATA

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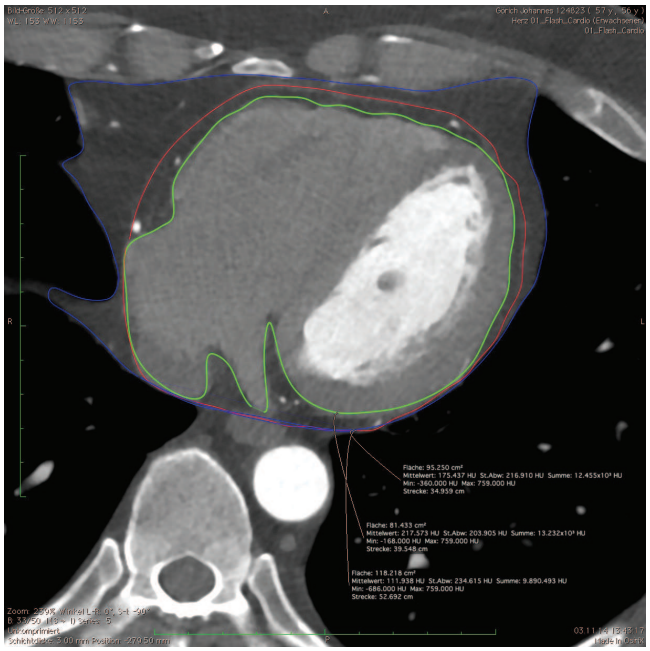
Recently, the significance of blood vessel- and heart-associated adipose tissue (epicardial, pericardial, periadventitial and intramyocardial) in the pathogenesis of atherosclerosis is increasingly pursued both at experimental and clinical level (1, 2). As a part of scientific collaboration between the Medical University in Varna and the Radiological Center in Heidelberg asymptomatic patients are examined for risk assessment of coronary artery disease. The investigations are carried out with a new spiral CT (computed tomography) Twin Beam Dual Energy technology CT force. In contrast to the conventional CT devices, the heart examination takes place in a very short time (0.15 sec.), in low dose technology with reduction of radiation exposure by 90 % (0.1 to 0.6 ms) and in a very high spatial and temporal resolution (0.25 mm / 160 ms). Since this novel imaging technique allows the very fast acquisition of more detailed parameters, this can be translated into more precise representation and quantification of pericardial and epicardial adipose tissue. We provide the first results of cardiac adipose tissue imaging with this technique. In just one examination sequence taking less than a second this method provides calcium score by CT angiography of the coronary arteries as well as the three-dimensional morphology of the epicardial adipose tissue.



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DIFFERENT ADIPOGENIC POTENTIAL OF MESENCHYMAL STEM CELLS ISOLATED FROM LIPOMA AND NORMAL ADIPOSE TISSUE - A PRELIMINARY RESULTS

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Lipoma is a benign adipose tissue tumor that most frequently appear in the subcutaneous tissue. It has been shown that adipose-derived stem cells (ADSCs) isolated from lipoma tissue (LDSCs), exhibit characteristics similar to ADSCs isolated from normal adipose tissue. They are morphologically similar, express characteristic mesenchymal stem cell markers and have the potential to differentiate into multiple lineages such as adipocytes, osteoblasts and chondrocytes. The aim of our study was to examine whether LDSCs have the same potential to differentiate into adipocytes as ADSCs.

Tissue samples were excised from the subcutaneous tumor tissue or normal subcutaneous adipose tissue during surgery. Cells were isolated by enzymatic digestion and cultured in standard cell culture conditions. After second passage both LDSCs and ADSCs were subjected to adipogenic differentiation for 21 day. As negative control, cells were grown in standard cell culture media. Expression of characteristic mesenchymal stem cell markers, CD29 and CD44, was confirmed by immunocytochemical staining before subjecting to differentiation. Adipogenic differentiation was evaluated microscopically and by Oil Red O staining. **Results:** Both LDSCs and ADSCs were morphologically similar after isolation and were positive for CD29 and CD44 mesenchymal stem cell markers. We have shown that LDSCs have weaker potential to differentiate into adipocytes than ADSCs. After 21 day of differentiation, both LDSCs and ADSCs were phenotypically different from cells that were grown in standard cell culture media and had characteristic shape, but there were less mature adipocytes filled with lipid droplets in LDSCs cultures than in ADSCs cultures. We can conclude that, in these conditions, LDSCs have weaker potential for differentiation toward adipocytes than ADSCs. This finding may contribute to the characterization of lipoma and explanation of the mechanisms of its formation, although further investigation on molecular level is needed.

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. III 41017)

FATTY ACID RECEPTORS AND BINDING PROTEINS IN PROGENITOR CELL NICHE OF ADULT PRIMATE BRAIN

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The adult brain harbors neural stem/progenitor cells (NPCs). They exist in microenvironments known as progenitor cell *niches*. The subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) along the anterior horn of the lateral ventricle are the two best described niches in the mammalian brain. Here we describe some putative molecular signals expressed in these microenvironments of the adult monkey hippocampus. The G-protein coupled receptor 40 (GPR40) is a transmembrane receptor for free fatty acids, involved in insulin secretion in the pancreas. Interestingly, we found a molecular link between dietary signals such as free fatty acids and adult neurogenesis by means of the free fatty acid receptor GPR40 being expressed in the precursor cell niche. Further, we identified that fatty acid-binding proteins (FABPs), a group of molecules known to participate in cellular metabolic processes, were expressed in the hippocampal neurogenic zones and in particular in the niche astrocytes. Our results suggest that while a complex network of secreted and nuclear signals regulates neurogenesis in adult primate hippocampus, some of them are related to dietary signals. The k nowledge on the mechanisms regulating brain progenitors in adult primates would probably have an impact on future restorative therapies for human neurological diseases.

NERVE GROWTH FACTOR AND BRAIN-DERIVED NEUROTROPHIC FACTOR IN THE FAT FROM RATS WITH METABOLIC SYNDROME

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Obesity and the concomitant prevalence of metabolic syndrome (MS) and type 2 diabetes mellitus (DM2) had increased as a global epidemic during the last decades. The initial steps include low physical activity associated to high food consumption that increment circulating lipids and cytokines, and growth factors release by adipose tissue. This, in turn cause chronic inflammation and potentiation of insulin secretion, and insulin resistance.

Metabolic syndrome is a cluster of signs that increases the risk to develop DM2, cardiovascular diseases and certain types of cancer. The main signs of MS are central obesity, dyslipidemia, hypertension, hyperinsulinemia and insulin resistance. Depending on genetics, after a long period of hyperactivity, pancreatic beta cells become exhausted and DM2 overcomes.

Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF) are neurotrophic factors that are also synthetize and liberated by white adipose tissue. We have previously observed that NGF is also produced and secreted by pancreatic beta cells, and increases insulin secretion; it is then possible that in obesity there is more NGF in extracellular media that could contribute to hyperinsulinemia.

In this work we analyzed NGF plasma levels and NGF produced by peripancreatic (Ppf) and epididymal (Epf) fat during the development of MS. We also evaluated BDNF produced by the fat. We developed MS in Wistar adult male rats (2 months old) by feeding experimental rats with high sucrose (20%) in the drinking water, for 2 and 6 months and compared to controls.

After two months, treated rats developed MS, characterized by central obesity, mild hypertension, hypertriglyceridemia, hyperinsulinemia and insulin resistance (MS rats). This signs were also present after 6 months of treatment; interestingly in the later they also showed fasting hyperglycemia.

Moreover, Ppf and Epf fat from MS rats, showed higher amounts of NGF than control rats, after 2 and 6 months of treatment. The highest level of NGF was observed in Epf at 2 months of treatment. Interestingly, in both treatments types, NGF amount in Epf was higher than in Ppf. The amount of BDNF significantly increased in 6 months MS rats, in both adipose depots, compared to controls. Finally we also analyzed the fat mRNA expression pattern of some cytokines in fat from MS rats (6 months of treatment). We found a significant increase in the expression of IL6 in Ppf and Epf, and IL10 in Epf, and a significant decrease in resistin in Ppf.

Our results demonstrate adipose changes of NGF, BDNF, IL6, IL10 and resistin that could be important in the biology of adipose tissue during obesity and metabolic syndrome, and probably in the physiology of pancreatic beta cells, and insulin secretion.

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OBESE PATIENTS AS A CHALLENGE FOR PERSONALIZED MEDICINE: EXAMPLES FROM DRUG METABOLISM AND TOXICITY

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The good physician cares for the disease;
the great physician cares for the patient.
Sir William Osler (1849-1919)

The term „personalized medicine" is described as providing „the right patient with the right drug at the right dose at the right time." More broadly, personalized medicine may be thought of as the tailoring of medical treatment to the individual characteristics, needs, and preferences of a patient during all stages of care. In this respect obese persons are a real challenge to implement the new achievements of personalized medicine. In the present lecture some data will be given from the few examples for differences of drug distribution and metabolism and the resulting differences in drug effects and toxicity in obese persons. Some objectives for inconclusive results for personal pharmacotherapy in obese patients are that clinical trial protocols often specifically exclude obese patients, leaving the impact of obesity on the pharmacology of most drugs uninvestigated. *Obesity and drug distribution:* The main factors which affect the process of tissue distribution of drugs are their physiochemical properties, body composition, plasma protein binding and regional blood flow. An understanding of how the volume of distribution of a drug changes in the obese is critical, as this parameter determines loading-dose selection. Existing insufficient clinical data show that highly lipophilic drugs like benzodiazepines, tricyclic antidepressants, antibacterials (ciprofloxacin), anticoagulants (dalteparin), anticancer drugs (busulfan, cisplatin, docetaxel, vincristine), oral anticonceptives (norethisterone and ethinylestradiol with failed effects) and volatile anesthetics (halothane with increased liver toxicity; enflurane with increased risks for fluoride nephrotoxicity), IFN-α (lower serum concentrations), were distributed more extensively in adipose tissue of obese patients, therefore, loading dosage may need to be increased and in the same time some adverse reactions should be carefully monitored. *Drug protein binding* has not been demonstrated to be changed in obese persons. *Obesity and drug metabolism and clearance:* Drug clearance (CL) is the primary determinant to consider when designing a maintenance dose regimen. CL is largely controlled by hepatic and renal physiology. According to preliminary results of propofol, sufentanil and paclitaxel clinical studies, liver blood flow is likely to be increased in obese patients. The reviewed studies show that clearance of renally eliminated drug is higher in obese patients because of increased glomerular filtration and tubular secretion. Clinical data for changes in activity of various *drug metabolism enzymes* in obese patients are diverse: *CYP 3A4* – reduced metabolic activity to N-methylerythromycin, midazolam, triazolam, alprazolam and ciclosporin, alfentanil; *CYP 2E1* – increased metabolic activity to chlorzoxazone, volatile anaesthetics, including enflurane, sevoflurane and halothane, paracetamol (increased liver toxicity); *CYP 2D6* – increased metabolic activity to dexfenfluramine and nebivolol; *CYP 2A6* – increased metabolic activity to valproic acid, nicotine, methoxyflurane; *CYP 1A2* – slight increased activity to theophylline and caffeine; *CYP 2C9* – slightly increased activity to ibuprofen, phenytoin, and glipizide; *CYP 2C19* – higher activity to diazepam; *CYP 4A* – increased fatty acids oxidation; possible cause: enzyme genes expression is regulated by leptin receptor signaling. It is important to note that leptin treatment return to normal the changed CYP's activity in obese animals, the effect is due either to a direct second messenger event or to indirect changes in insulin, cortisol, and/or growth hormone levels. It should be proven whether this occurs in obese persons.

CHANGES OF THE HYPOTHALAMIC PROOPIOMELANOCORTIN AND AGOUTI-RELATED PEPTIDE CONTENT IN RATS TREATED WITH SIBUTRAMINE UNDER HIGH-CALORIE DIET

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Obesity is a major health and social problem that is characterized by high rate of distribution in the population. Under modern concepts the influence on eating behavior is one of the promising ways to correct the obesity. Both central and peripheral mechanisms are involved in energy metabolism dysregulation under obesity. Hypothalamus is the main regulation center of feeding behavior in the brain. Hypothalamus arcuate nucleus is the basic formation that regulates feeding behavior and contains two types of neuronal populations. The first ones contain agouti-related peptide (AgRP), that stimulates the food intake, the others - proopiomelanocortin (POMC), that inhibits the feeling of hunger. Sibutramine is an anorexigenic preparation that influences on the central cores of obesity development, but the mechanisms of influence on the feeding behavior are not fully understood. Therefore, the aim of our work was to study the effect of this preparation onto the AgRP and POMC content on hypothalamus of the rats fed high-calorie diet.

Adult male Sprague-Dawley rats weighing 250-300 g were used. They were divided into three experimental groups: intact animals (IG) that were fed a standard vivarium diet; rats that were fed high-calorie diet (model pathology – MP); rats that under high-calorie diet were administered sibutramine 10 mg / kg for 3 weeks (MP + sibutramine). The hypothalamus was removed, weighed and placed into 0.25 ml of 0.1 M acetic acid, boiled and dispersed tissue by sonication. Tissue homogenates were neutralized with 100 mM Tris (pH 7.0) and centrifuged at 14 000 rpm for 10 minutes. The AgRP and POMC content were determined in homogenate by radioimmunoassay kits (Phoenix Pharmaceuticals Inc., Mountain View, CA, USA).

The development of obesity was observed in rats fed high-calorie diet, that was accompanied by a significant increase of AgRP content (in 2.1 times) compared with animals from IG, conversely, the POMC content was significantly lowered (see table). AgRP and POMC are endogenous antagonists, so such dynamics under MP is natural and is mediated by insulin resistance and hyperleptinemia that accompany obesity. The normalization of AgRP and POMC content observed in rats administered sibutramine was revealed by correction of eating behavior in animals and the prevention of obesity under high-calorie diet. Sibutramine is an inhibitor of reverse neuronal uptake of serotonin and norepinephrine, and serotonin – in turn can stimulate the POMC secretion. In our previous studies we have shown the sibutramine inhibitory action on the neuropeptide Y effects, which acts synergistically with AgRP (Zagayko, 2015). In addition, sibutramine normalizes leptin and insulin levels under experimental insulin resistance (Derosa, 2010; Suliburska, 2012), and these hormones are inhibitors of the AgRP-containing neurons activity. This fact explains the AgRP decrease under sibutramine administration.

Neuropeptides	IG	MP	MP+ sibutramine
AgRP, pg/mg wet tissue	6.1±0.5	12.6±0.2*	7.3±0.2**
POMC, pg/mg wet tissue	3.2±0.1	1.9±0.05*	2.8±0.02**

* - statistically significant difference compared to the IG ($p \leq 0.01$);
** - statistically significant difference compared to the MP ($p \leq 0.01$).

Dynamics of the AgRP and POMC content changes indicates the sibutramine ability to correct obesity integrally, including normalizing of eating behavior, and as a consequence – the earlier feeling of satiety and prevention of overeating.

THE TRIO: ADIPOSSE TISSUE, IMMUNE SYSTEM AND ISLET B-CELLS

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The parallel increase in the prevalence of diabetes and obesity (recently dubbed diabesity) has been associated to the rapid environmental and social changes (1). The adipose tissue represents the main lipid depot, which is essential for the maintenance of the energy homeostasis of the human body. The immune system as a member of the defence system of the body's integrity is able to take part in the continuous surveillance of the "normal" adipose depots, reacting adequately to the smooth passing of the "quiet" (normal) adipocyte, to the "restless" adipocyte and mainly to the "aggressive" adipocyte as we characterized them previously (2). The adipocyte "aggressive" phenotype is able to produce and release a high number of adipokines consisting of cytokines and chemokines acting as *danger signals* (3, 4) perceived by the immune system (dendritic cells first) inducing so called *low grade inflammation*, with its multiple effects on the vascular endothelial cells in the heart, liver, kidney, brain, pancreatic islets, spleen, lymph nodes and other structures of the human body. By its big and dynamic secretory function, producing more than 200 various signaling molecules, the adipose tissue has been characterized as "the third brain" and "a tissue with high intelligence quotient" (5). A great part of adipokines are considered to be derived from the adipose stromal vascular cells, explaining the great plasticity of this huge secretory organ. Apart, adipose macrophages and dendritic cells as well as other immune cells (T and B lymphocytes, and mast cells) can also interact with adipocytes. Notably, the dendritic cells (6) are present mainly in the omentum (uniquely mammalian structure) where, close interaction take place between the adipocyte layers up to 10 mm surrounding the lymph nodes. In interesting cooperation, the adipocyte closer with lymph notes gain some lymphoidic phenotypes whereas, the external cell layer of lymph nodes gain some metabolic phenotype of the adipocyte (7). A similar behavior can be observed in the various compartments of the adipose tissue: perivascular, epi- and pericardial, peripancreatic, intra- and perimuscular, etc. The association between obesity ant type 2 diabetes mellitus shapes an "old couple" (8). The pathogenic link between the adipose tissue and pancreatic β-cell is insured by the proinflammatory reaction triggered by the adipokines TNF-α, IFN-γ, IL-6, MCP1, CCR2, including a decrease in adiponectin and NGF secretion (5), all released by the aggressive adipocyte (9).

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A VICIOUS CONNECTION: ADIPOSE TISSUE-OBESITY-IMMUNE SYSTE-ATHEROSCLEROSIS

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The cell constituents of the adipose tissue secrete a large number of bioactive adipokines that have endocrine, paracrine and autocrine effects. Adipocytes and adipose tissue-derived macrophages are the source of the several circulating mediators of inflammation. Excess or unbalanced secretion of adipokines, a frequent occurrence in obesity, induces an inflammatory state that in turn contributes to obesity-associated cardiovascular diseases. Atherogenesis occurs irrespective of the presence of dyslipidemia (conventional view) and inflammatory cells have a key role in atherosclerosis that is now recognized as a subacute inflammatory condition of the vessel wall. Atheroma formation is a continuous process arbitrarily delineated by consecutive stages.

Stage I. Commencement of plaque formation: modulation of endothelial cells (EC) constitutive functions: a. loss of EC controlled permeability, increased transcytosis and intimal deposition of plasma LDL; b. changes in EC phenotype, i.e. the switch toward a secretory type; c. alteration of EC net negative surface charge.

Stage II. EC dysfunction and the initiation of a multipart inflammatory process manifested by EC "activation": alterations of the EC non-adhesive and non-thrombogenic surface, expression of plasmalemmal new or additional cell adhesion molecules or inflammatory mediators. EC activation and dysfunction, is a defence reaction assisting the vascular endothelium to recruit specifically blood inflammatory cells.

Stage III. Robust inflammatory reaction: adhesion and extravasation of monocytes and lymphocytes, fatty streak formation: a. Adhesion, diapedesis and intimal residence of pro-inflammatory monocytes; platelets assist the recruitment of blood monocytes; b. Recruitment of circulating T-Lymphocytes ($CD4^+$T cells) together with antigen-presenting dendritic cells in response to chemokines and chemoattractants. Upon recognition of an antigen, the type 1 helper T cells (Th1) become activated, secrete cytokines and cell surface molecules, which contribute to macrophage activation and potentiation of the inflammatory response; c. PMN release superoxide and mediators that at the blood–vessel wall interface amplify recruitment of inflammatory cells and within the plaque contribute to its vulnerability.

Stage IV. Fibrous plaque formation: inflammatory cells send molecular messages that govern the plaque development, including the clonal accumulation of SMC from the media to the intima or from other sources (circulating bone marrow cells and vascular progenitor cells from artery adventitia). Migrated SMC switch to a secretory phenotype synthesising a hyperplasic basal lamina and matrix.

Stage V. Calcified atherosclerotic fibro-lipid plaque: formation of a lipid rich necrotic core encapsulated by fibrous tissue. Excess extracellular unesterified cholesterol nucleates into cytotoxic crystals; plaque evolves to complicated atheroma.

Stage VI. Complicated plaque: rupture, thrombosis. EC covering the fibrous cap become thin and loaded with lipid droplets (EC- derived foam cells) and are prone to rupture. *Macrophages* infiltrate the fibrous cap and secrete inflammatory cytokines and MMPs. *SMC* decrease collagen synthesis. *Mast cells* secrete tryptase and chymase that assist in plaque destabilization and atherothrombosis. All the cells (resident or emigrated), every chemokine, cytokine, MMP and vasa vasorum (that contribute to plaque formation) are targets for atherosclerosis therapy.

Targeted suppression of pro-inflammatory mediators in adipocytes may cut the vicious connection between adipose tissue-obesity-inflammation and atherosclerosis and help to prevent or retard plaque development.

MITOCHONDRIAL DYNAMICS: NOVEL PERFORMANCE IN DIABETIC MYOCARDIUM

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Nowadays we witness a revitalization of research on mammalian mitochondria, along with disclosure of a plethora of novel functions of these organelles, including their key role in cellular survival and death. Mitochondria are abundant within cardiomyocytes occupying at least 30% of cellular volume and providing energy (ATP) for efficient contraction of the heart; any modification of membrane potential, of permeability transition pores opening, of mtDNA structure, or the aberrant interactions with nucleus, intracellular lipid deposits, or sarcoplasmic reticulum will generate a diminished bioenergetic reserve associated with impairment of heart contractility. The discussion links the electron microscopy evidence on mitochondrial dynamics within diabetic left ventricular coronary endothelium and cardiomyocytes to the newly identified molecules/mechanisms beyond it. The issues examined are: (*i*) the active bidirectional redox communication between endothelial cells and cardiomyocytes in diabetic myocardium, (*ii*) the intracellular events associated with mitochondrial dynamics, such as the fusion of healthy mitochondria with dysfunctional ones aiming preservation of cellular energetic potential, the "quality controller" role of mitochondria consisting in removal of deficient fragments (by fission) and their elimination *via* a specific form of autophagy (mitophagy), the inter-mitochondrial exchange of membrane and matrix proteins (by "kissing" and "nanotunneling"), (*iii*) the "retrograde signaling pathway" from mitochondria to the cytosol and the mitochondrial cross talks with nucleus, lipid droplets, and sarcoplasmic reticulum, and (*iv*) a perspective of mitochondrial medicine in diabesity, i.e. diabetes linked to obesity. Modulation of mitochondrial dynamics to prevent or treat diabesity emerges as a novel strategy with potential for cardioprotection in translational medicine.

METABOLIC SYNDROME: A CAUSE OR A CONSEQUENCE OF NONALCOHOLIC FATTY LIVER DISEASE?

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The metabolic syndrome (MetS) is an independent risk factor for nonalcoholic fatty liver disease (NAFLD). Moreover, NAFLD is associated with a high risk of severe liver fibrosis. The prevalence of the MetS increases with increasing BMI, from 18% in normal-weight NAFLD subjects to 67% in obese subjects. MetS is quite common in NAFLD patients; they are strongly associated, but it remains unknown whether NAFLD is a cause or effect of MetS; NAFLD predicts type 2 diabetes and cardiovascular disease (CVD); it is important to identify this subset of NAFLD patients with MetS because they frequently have more advanced histology and also have a heightened risk of CVD. Nonalcoholic fatty liver disease is the most common liver disorder; it is an important cause of liver-related morbidity and mortality; NAFLD covers a range from simple steatosis (NAFL) to nonalcoholic steatohepatitis (NASH); NASH may progress to cirrhosis and end stage liver disease; NAFLD is the most common cause of hepatocellular carcinoma; NAFLD occurs in 9% of overweight patients and 21-33% of those with morbid obesity. Ultrasonographic surveys of the general population indicate the presence of fatty liver in 16-25% of adults in the United States. Metabolic syndrome is an important risk factor for CVD, as well as all-cause mortality; because of the close association between the MetS and NAFLD, there has been great interest in determining the actual cardiovascular risk in patients with NAFLD. NHANES study reported that persons with elevated serum ALT levels in the absence of viral hepatitis or excessive alcohol consumption had an increased CVD risk. Some atherogenic mechanisms related to NAFLD contributed to the excess CVD risk beyond what can be explained by the MetS. NAFLD is now considered a chronic inflammatory condition and thus adds further atherogenic stimuli to the already high oxidative and proinflammatory status conferred by the MetS.

Metabolic syndrome in Romania, PREDATORR Study (2014): the prevalence of MetS in adult population of Romania (20-79 years) is 38,44% (more than six millions adult subjects).

METABOLIC SURGERY'S EFFECTS ON END ORGAN DAMAGE

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During the last over 60 years, surgery has demonstrated to be very effective treatment for weight loss and glycemic control. The different names associated with this selected category of surgical procedures - weight loss surgery (WLS), obesity surgery, bariatric (BS) or metabolic surgery (MS) - were expressing the level of understanding, using and focusing its effects for the patients' morbidities control. Today, MS has proven its long term success in contrast to conservative treatment options and this is well documented in terms of weight loss, improvement or disappearance of comorbid diseases and even in gain in quantity of life. Moreover, the introduction of laparoscopy, of Bariatric Surgery Programs and Centers of Excellence (COEs): decreased the perioperative morbidity and the postoperative evolution is substantially improved. The IFSO –EC COEs Register (2015) demonstrates a very low peroperative surgical risk: *Per-operative complications* (1.09%) 238/21.838; *post-operative complications* (2.54%) 546/21.506 *Post-operative mortality* (≤ 30 days) (0, 02%) 4/21.838; *Late mortality (>30 post-op days)* 13/21.838 (0.06%). In Ponderas Hospital COE, the postoperative mortality was nil (0/5274). Demonstrating its efficiency and safeties, MS is considered by the most Associations of Diabetes as a surgical options for adults with BMI ≥ 35 kg/m2 and type 2 diabetes, especially if the diabetes is difficult to control with lifestyle and pharmacologic therapy. As a next step MS focuses to prevention /remission or control of the morbid consequences of obesity/T2DM In this paper is presented the evidence on the efficacy of MS/BS on prevention, delay, or even reversal of end-organ damage for: cardiovascular disease, diabetic kidney disease, diabetic retinopathy, NAFLD, reproductive function and fertility as well on its influence on malignancies. Currently BS/MS remains the only treatment offering predictable, long term effective weight loss and therefore will play a key role in minimizing, stabilizing and/or improving end organ damage in obesity.

PARTICULARITIES IN APPROACHING THE HYPERTENSIVE PATIENT WITH OBESITY

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The hypertension-obesity association is frequently encountered in daily clinical practice, more than 60% of hypertensive patients being obese and requiring a special approach. Obesity can impose some difficulties in defining the presence and control of elevated blood pressure values (stressing the need of using adequate cuffs when measuring blood pressure), and may be a cause of resistance to antihypertensive treatment. In Romania, according to SEPHAR II data, the prevalence of obesity among hypertensive patients vary depending on the method used to define obesity from 42.5% (by body mass index, BMI) up to 60.7% (by waist circumference). The most commonly used index for defining obesity is BMI, which is expressed as body weight (kg) to height (m^2) ratio. At population level, higher BMI values are associated with a greater prevalence of co morbidities such as type 2 diabetes and cardiovascular disease. However, in clinical practice, equally obese individuals may or may not be characterized by the expected co morbidities of obesity. Other method used in daily practice is the measurement of subcutaneous fat by skin fold calipers. Both methods enable us to evaluate the total body fat. Visceral obesity (or abdominal obesity) which can be defined by waist circumference > 102 cm in males and > 88 cm in females (according to the United States National Cholesterol Education Program's Adult Treatment Panel III), or measured by computer tomography is now recognized as an Independent Predictor of All-Cause Mortality. The ethiopatogenic link between obesity and hypertension relies on that fact that adipose tissue is an important endocrine organ and a site of production for inflammatory cytokines (such as interleukin-6 and tumor necrosis factor-α) and a potentially protective cytokine (such as adiponectin, which is reduced in visceral obesity). Leading to renin-angiotensin system (RAS) stimulation, sodium reabsorption, insulin resistance, endothelial dysfunction and sympathetic nervous system (SNS) activation, obesity can trigger the increase in blood pressure values. Also by favoring obstructive sleep apnea (OSA), obesity may also lead not only to hypertension but also to resistance to antihypertensive treatment if not addressed properly by therapy. It becomes clear that treatment approach of hypertensive patients with obesity must be exhaustive. First of all weight loss must be the Primary treatment goal as it may improve not only blood pressure (each 1 kg body weight reduction, decreases blood pressure by 1.05/0.92 mmHg), but also the metabolic components and delay diabetes onset. This may be achieved by reduced-fat and reduced-carbohydrate hypocaloric diets and by physical exercise and only in restricted cases by use of weight loss drugs (orlistat). In patients with morbid obesity, bariatric surgery remains the optimal way to achieve proper weight loss. Regarding antihypertensive drugs, mono-therapy is seldom sufficient to control blood pressure. Trying to identify reasonable first choices drugs, "…as MS can be considered a pre-diabetic state, antihypertensive agents potentially improving or at least not worsening insulin sensitivity such as RAS blockers and calcium antagonists, should be considered as the preferred drugs". Especially in these types of patients the doctors must consider metabolic side-effects of antihypertensive drugs (especially for beta-blockers and thiazides). In case of resistance to treatment, central sympatholytic drugs are another treatment option (obesity being characterized by increased SNS tone). Also active screening for OSA in these patients is mandatory and if detected OSA treatment strategies - upper airways surgery, mandible protruding devices, and continuous positive airway pressure must be applied alongside antihypertensive treatment, as effective treatment of OSA, may reduce cardiometabolic risks. Likewise, renal sympathetic denervation could substantially reduced blood pressure in patients with real treatment-resistant hypertension and may also improve glucose metabolism.

RISK OF LIVER CANCER IN NONALCOHOLIC STEATOHEPATATIS

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Hepatocellular carcinoma (HCC) is the only digestive cancer with a continuous increase of incidence, prevalence and mortality. The mortality to HCC has increased by more than 50% in the last two decades and remains as one of the cancer with the highest mortality ratio to incidence (0.95). So, HCC is now the second most frequent cause of death from all digestive cancers, after pancreatic cancer. Also the lethality of HCC is highs (0.9), very few new cases survive over one year.

Variables	OR (95% CI)	p value
PNPLA3 736409 genotype	2.26 (1.23-4.14)	0.0082
Age	1.24(1.17-1.32)	<0.0001
Sex (male)	11.11(4.17-33.33)	<0.0001
BMI	0.94(0.87-1.02)	0.148
Diabetes	2.33(0.93-5.81)	0.070
Cirrhosis	9.37(3.82-23.00)	<0.0001

***Table 1**. Multivariate analysis of the effect of PNPLA3 genotype on NAFLD related HCC risk (see 1).*

Recent epidemiological data suggest a causal relation ship between NASH and HCC. Many mechanisms are involved in HCC pathogenesis in nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): oxidative stress, inflammation and metabolic disarray, the most important risk factors being PNPLA3 genotype age, male, gender, diabetes mellitus, obesity and liver cirrhosis (Table1). Hepatocellular carcinoma could be present even in non-cirrhotic NAFLD and NASH. There are no drugs which could prevent HCC in these liver diseased. Early HCC detection is performed by abdominal ultrasound and alpha-feto-protein every 4 month in the high risk populations as cirrhotic, obesity and diabetes patients, also older males.

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THE IMPORTANCE OF A HEALTHY FOOD, WATER AND ENVIRONMENT FOR THE DEVELOPMENT AND PERFORMANCE OF THE HUMAN BRAIN

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The authors debate the major actual problem of healthy nutrition and its impact on the development of the nervous system referring mostly to young persons and the intrauterine period. The modifications of the human genome through alimentation, water and environment can be in such circumstances unpredictable and irreversible. The development of the human body depends mostly on genetic factors. These in turn depend on a series of nutritional factors, environmental factors and possible nociceptive external aggressions. The maximal teratogenous period stretches from the 2nd week of pregnancy until the 30th week, with the brain being the most sensitive organ prone to lesions. The nervous cell is highly sensitive to teratogenous agents. Nutriments need to be chosen carefully to allow the best course of development of the organism and especially the nervous system. Young organisms until the age of 20 need to be protected from poisonous factors from food and the external environment. Studies from all over the world have demonstrated that food and water influence the genotype and the fenotype in a decisive way being the source or vector of a various array of diseases. The consequences of "industrial alimentation" enriched with growth hormones and other alimentary additives are shown in this study together with the poisonous effects of alcohol consumption, tobacco, drugs, radiation etc. Furthermore the authors bring in discussion quantum mechanics and physics studies that aim to show that all human beings are interconnected and that each one of us has the potential to influence his partners by what he thinks. At the end the authors insist upon alimentation issues promoting natural food and presenting in parallel the risks and possible consequences of genetically modified food. The authors advocate against mall culture and industrial alimentation while presenting the advantages gained by natural alimentation and the use of several well known herbal remedies. Also, the authors wish to emphasize the proper hydration through natural water (unaltered industrially, chemically or carbonated) and the external elements which influence the cerebral activity (stress, smoking, alcohol and environment). All these constellations must be obeyed as much as possible in order to obtain a proper harmonious cerebral development for young persons and also for the preservation of the brain activity in mature persons. Given the very large metabolic needs of the human brain, the healthy nutrition is a crucial factor in the brain development especially in the intrauterine period and early childhood together with the influence of external factors and, intriguingly, the development of gut microbiota (1).

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GENETIC FACTORS INVOLVED IN THE PATHOGENESIS OF HUMAN OBESITY

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Obesity is one of the most common chronic diseases in human populations across the globe, the current epidemic affecting over 500 million adults. Similar to other common human complex diseases obesity pathogenesis involves both genetic and environmental factors. The last include hypercaloric diet, sedentarism, stress and other conditions such as urbanization and westernized lifestyle. Considerable evidence from epidemiologic twin, adoption and family studies indicated that body weight and body fat disposition have a heritability of up to 70%, highlighting the importance of heredity/genetics. The discovery of single gene variants associated with rare causes of extreme obesity in children highlighted the pivotal role of hormonal and neural hypothalamic networks regulating body weight. The majority of these genes were discovered following studies in animal models (mice) and subsequently confirmed in humans. Among these are the genes for leptin (Lep), leptin receptor (LepR), proopiomelanocortin (POMC), melanocortin 4 receptor (MC4R) and prohormone convertase 1 (PCSK1). Some other gene variants were discovered analyzing the equally rare cases with complex syndromes associating obesity. Again most genes identified are related to central nervous system appetite centers. In contrast with the severe obesity cases induced by homozygous mutations in these genes, carriers of heterozygous mutations exhibit less severe obesity. In contrast with monogenic forms of obesity, in the common form of polygenic obesity the genetic risk is influenced by the combined effect of variation at numerous loci. Thus, the recent genome-wide association studies for obesity related traits such as body mass index or waist circumference identified more than 120 gene variants/loci, the vast majority with a modest effect (their combined effect explains only a small part of BMI heritability). Many of these were shown to be highly expressed in the brain, particularly in the hypothalamus, highlighting again their importance in regulating food intake and, subsequently, adiposity. The fat tissue and obesity associated gene (FTO) on chromosome 16 was the first gene convincingly proven to be associated with common human obesity. The fact that gene variants identified so far has such a poor predictive value for obesity, especially when compared with risk calculators based on clinical factors, is known as missing heritability and has several potential explanations. Epigenetic factors (such as DNA methylation and histone modification) presumably play an important role in the pathogenesis of human obesity. They might mediate the effects of the environment on the risk of obesity. Further research is needed to clarify the role of genetic variation and epigenetic mechanisms in the development of human obesity.

PHARMACOLOGICAL INHIBITION OF HISTONE DEACETYLASE REDUCES OXIDATIVE STRESS AND INFLAMMATION IN THE AORTA OF DIABETIC MICE

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Hyperglycaemia-induced functional and structural alterations of the vascular wall in diabetes are partially mediated by oxidative stress generated by the activated NADPH oxidase (Nox). Hitherto, the molecular mechanisms that accounts for Nox upregulation are not entirely elucidated. In this study we hypothesize that histone acetylation has a role in the regulation of Nox and that histone deacetylase (HDAC) inhibition may have the potential to counteract the vascular oxidative stress and inflammation in diabetes. Male C57BL/6J mice were rendered diabetic with streptozotocin. The animals were distributed into four experimental groups to receive vehicle or suberoylanilide hydroxamic acid (SAHA), a selective HDAC inhibitor, every other day for four weeks: (*i*) non-diabetic + vehicle, (*ii*) non-diabetic + SAHA, (*iii*) diabetic + vehicle, and (*iv*) diabetic + SAHA. Lucigenin-enhanced chemiluminescence assay, real-time PCR, and Western blot analysis were employed to investigate vascular epigenetic changes, Nox regulation, and the expression of pro-inflammatory markers. Western blot analysis revealed that the HDAC1 and HDAC2 protein expression levels were significantly elevated in the aorta of diabetic mice compared to non-diabetic control animals. Treatment of diabetic mice with SAHA greatly reduced the augmented Nox activity and the gene and protein expression of the Nox1, Nox2, and Nox4 subtypes. Pharmacological targeting of HDAC generated vascular anti-inflammatory activities as illustrated by the inhibitory effects of SAHA on intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and monocyte chemoattractant protein-1 gene expression in the aorta of diabetic mice. In conclusion, pharmacological inhibition of HDAC reduced Nox expression and the ensuing reactive oxygen species formation in the aorta of diabetic mice. These data indicate the existence of a new epigenetic mechanism whereby changes in chromatin conformation leads to Nox upregulation and inflammation in vascular cells in diabetes.

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GLUCOCORTICOIDS UPREGULATE APOLIPOPROTEIN E GENE EXPRESSION IN A MACROPHAGE-SPECIFIC MANNER

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Apolipoprotein E (apoE), a glycoprotein involved in the lipoprotein metabolism, is synthesized by the liver and other peripheral sources. Macrophage-derived apoE plays an atheroprotective role, while its overexpression in the liver causes hypertriglyceridemia. The aim of our work was to investigate the glucocorticoid induced cell-specific mechanism of apoE gene regulation. ApoE gene expression in mouse peritoneal macrophages, RAW 267.4 and HepG2 cells treated with the ligand (dexamethasone) or antagonist (mifepristone) of glucocorticoid receptor (GR) was tested by Real-Time PCR using TaqMan probes. The effect of short-term dexamethasone treatment on apoE gene expression in macrophages and liver of C57BL/6J mice was also evaluated. The capacity of ligand-activated GRs to enhance the apoE promoter activity was assessed by transient transfections using plasmids encoding the apoE proximal promoter and GRα expression vectors, in the presence of dexamethasone. GR binding on the apoE promoter was tested by DNA pull-down assays and chromatin immunoprecipitation experiments. Real-Time PCR experiments showed that dexamethasone treatment induced a 5-6 fold increase in apoE mRNA levels in RAW 264.7 macrophages and mouse peritoneal macrophages, but not in hepatocytes. Mifepristone treatment resulted in a significant reduction of apoE expression in macrophages. One week dexamethasone administration to C57BL/6J mice increased apoE mRNA levels in macrophages, but not in liver. Dexamethasone-activated GRs specifically increased the activity of apoE promoter in macrophages. DNA pull-down assays showed that GRs bind to -115/-65 region of the apoE promoter and chromatin immunoprecipitation indicated that GRs are recruited to the apoE promoter following dexamethasone treatment of cells. Taken together, our data revealed a differential effect of glucocorticoids on apoE gene expression, in macrophages and hepatocytes. Despite that GR binding site (located in the region -115/-65 of the apoE promoter) is functional in both cell type analyzed, the glucocorticoids regulate the apoE gene expression in a macrophage-specific manner. These data may contribute to the identification of specific drugs increasing the apoE level selectively in macrophages, to stimulate the cholesterol efflux from the atherosclerotic plaque.

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IMPROVEMENT OF THE METABOLIC PROFILE AND PREVENTING ATHEROSCLEROSIS IN OBESE CHILDREN BY USING SOME SUPPLEMENTS

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It was demonstrated that omega-3 fatty acids supplements and sea-buckthorn (*Hippophae rhamnoides*) pulp oil obtained by cold pressing have antiinflammatory effects and improve the lipid profile in obese adults. Less studies were done in childhood obesity. The aim of this study is to investigate the beneficial effects of the supplements in obese children. In our studies done in 59 obese children (10-16 years old) versus 30 healthy controls we measured blood parameters before and after three months of treatment either with 323 mg/day of omega-3 fatty acids (EPA, DHA) associated with vitamin A 200 μg, vitamin D 1.25 μg, vitamin E 2.5 mg and vitamin C 30 mg or with 800 mg/day Sea-buckthorn pulp oil. Spectrophotometric and ELISA methods were used. Usual plasma variables (glycaemia, creatininemia), antropometric parameters, plasma triglycerides, inflammatory and oxidative stress markers, adipokines, plasma minerals, insulin resistance markers, antifibrinolitic factor PAI-1 and preatherosclerotic marker (carotid intima media thickness) markers were improved significantly after treatment. In conclusion, omega-3 fatty acids and sea-buckthorn pulp oil supplements are highly recommended in obese insulinoresistent children.

ADIPOKINES PROFILE AND CNR1 POLYMORPHISMS IN METABOLICALLY UNHEALTHY LEAN AND METABOLICALLY HEALTHY OBESITY PHENOTYPES

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Metabolically healthy obese and metabolically unhealthy lean are two atypical metabolic phenotypes whose characteristics and prognosis are a subject of extensive scientific debate. The endocannabinoid system may contribute to the association of fat accumulation with metabolic disorders. The aim was to assess the prevalence of different metabolic phenotype and to analyze the adipokines profile, insulin resistance and five single nucleotide polymorphisms of endocannabinoid type 1 receptor (CNR1) in metabolically healthy obese and metabolically unhealthy lean phenotypes. We performed a cross-sectional analysis in a random sample of 507 individuals (52.9±14 years, 47.1% male) which were classified as metabolically healthy lean (MHL) or overweight/obese (MHO) and metabolically unhealthy lean (MUHL) or overweight/obese (MUHO), based on the body mass index (BMI) and presence of metabolic syndrome. Single nucleotide polymorphisms of CNR1 (rs12720071, rs806368, rs1049353, rs806381, rs754387) were genotyped. The frequency of MHO, MUHL and MUHO phenotypes was 16.2%, 6.9% and, respectively 58.6% among all participants. MHO exhibited significantly lower leptin levels [MHO: 11.7 ng/ml (interquartile range 3.8-17.4) vs MUHO: 14.9 ng/ml (interquartile range 6.7-24.6) *versus* MHL: 4 ng/ml (interquartile range 1.5-15.5)], insulin levels [MHO: 9.4 µUI/ml (interquartile range 6.8-12.2) vs MUHO: 11.6 µUI/ml (interquartile range 8.3-15.7) *versus* MHL: 6.6 µUI/ml (interquartile range 3.9-10.3)] and HOMA-IR [MHO: 2.6 (interquartile range 1.7-3.4) vs MUHO: 3.4 (interquartile range 2.4-4.7) *versus* MHL: 1.6 (interquartile range 0.9-2.6)] than MUHO participants, but significantly higher than MHL participants. MUHL participants had similar adipokines profile but significantly higher HOMA-IR [2.2 (interquartile range 1.5-4)] than MHL. Leptinemia, insulinemia and HOMA-IR were significantly lower in MUHL than in MUHO subjects. Expression of CNR1 genes showed a similar alteration pattern in all metabolic phenotypes. Multivariate logistic regression analysis revealed age, HOMA-IR [OR 24.3 (CI 6.2-95.2), $p < 0.001$], adiponectin [OR 0.9 (CI 0.9-0.99), $p = 0.04$] and insulin [OR 0.5 (CI 0.3-0.6), $p < 0.001$] as independent predictors of MUHL phenotype. HOMA-IR [OR 12.3 (CI 3.2-47.1), $p < 0.001$], insulin [OR 0.6 (CI 0.4-0.8), $p = 0.0012$], leptin [OR 1.1 (CI 1.1-1.2), $p = 0.02$] and adiponectin [OR 0.9 (CI 0.9-0.98), $p = 0.01$] were independent predictors of MHO phenotype. The mutant G allele (GG+AG) of rs12720071 reduces the odds of having MHO phenotype by approximately 80% (p = 0.02). MHL was considered the reference category in regression analysis. The current study provides evidence that MHO subjects had more impaired adipokines profile and were less insulin sensitive than MHL subjects, thus the MHO concept should be applied with caution. Insulin resistance and adverse adipokines profile were less frequent in MHO and MUHL than in MUHO subjects. Only rs12720071 has been identified as a modulator of the MHO phenotype.

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THE SYNERGY BETWEEN ADIPOSE TISSUE GRAFT, ADSC, LASER AND PRP IN THE CONTEXT OF REGENERATIVE PLASTIC SURGERY

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Surgical techniques based on transfer of autologous fat, adipose-derived stem cells (ADSC), ablative surgical LASERS and PRP have among other effects a strong biostimulative tissue regenerative outcome. They are becoming part of newly appeared plastic regenerative surgery (PRS). Each component of our approach has its own advantages when used alone, but when used in combination there are clinical and histological evidences that the synergistic effect gives an empowered result which is superior to each of individual component result. This is due to the stem cells' and other cells' activating and healing potential under the stimulative and complementary effect of LASER and PRP. Nowadays, in our practice, we are currently offering treatments of PRS. By the time we refined and enriched our techniques and equipment. Since 2008, in Plastic Surgery Department of ProEstetica Medical Center, over 300 patients underwent single at the beginning and then combined regenerative closed surgery based on the following methods introduced gradually: 1. Fat transplant (micrografts) and nanografts since 2013. When necessary, fat removal by fine lipoaspiration was done. 2. LASER assisted lipolysis (LAL) with optical fiber (DIODE LASER λ = 980 nm) since 2008. Fractional CO2 LASER (λ = 10600 nm) for resurfacing and fat graft stimulation since 2008. 3. PRP treatment (injections and spreading) since 2012. 4. Additional ADSC standardized enzymatic digestion from lipoaspirate since 2013. Autologous fat transplant proved to be an important regenerative clinical application due to is composition of progenitor (stem) cells and other regenerative factors. From clinical experience and research findings we arrived to the following conclusions: 1. The ADSC lead to better results when combined with fatty tissue (enriched AFT). 2. The fat graft has a better survival rate and less complications when is enriched with ADSC (ex: in breast correction). 3. Additional LASER treatments, especially fractional CO2 LASER application is optimizing the regenerative activity of ADSC and autologous fat graft. Taking in consideration the clinical observations we started to learn more through research about optimization effect over fat and ADSC when LASER exposure was performed. 4. Growth factors and cytokines administrated simultaneously with ADSC and AFT are also optimizing factors in PRP.

OXIDIZED LDL INDUCE THE EXPRESSION OF PRO-INFLAMMATORY MOLECULES IN LIPID-LOADED MACROPHAGES

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Oxidation of plasma proteins and lipids, including low density lipoproteins (LDL), contributes to accelerated atherosclerosis. The oxidative processes alter the proper functioning of the endoplasmic reticulum (ER), the site of protein and lipid synthesis, resulting in ER stress which activates the inflammatory pathways in macrophages. The aim of this study was to evaluate whether exposure of human macrophages in culture to oxidized LDL (oxLDL) stimulates the secretion of pro-inflammatory molecules, such as C-reactive protein (CRP) and matrix metalloproteinase-9 (MMP-9) and if oxLDL-induced ER stress and oxidative stress determine the secretion of CRP and MMP-9 from lipid-loaded macrophages. Oxidized LDL were prepared by incubation of native LDL (nLDL) with 10μM $CuSO_4$ at 37°C for 24 h, dialysed extensively, kept under sterile conditions and used within 2 weeks. Cultured THP-1 macrophages were incubated with oxLDL, in the presence or absence of inhibitors of ER stress (sodium phenylbutyrate, PBA) or oxidative stress (N-acetyl cysteine, NAC, and apocynin, a NADPH oxidase inhibitor). The following parameters were determined: (*i*) free cholesterol (FC), total cholesterol (TC), 7-ketocholesterol (7-KC) by gas chromatography-mass spectrometry GC/MS/MS; (*ii*) ER stress markers, NADPH oxidase activity and reactive oxygen species (ROS) production by Western blot, chemiluminescence and spectrofluorimetric techniques; (*iii*) secreted CRP and MMP-9 by Western blot, and (*iv*) secreted MMP-9 activity by zymography. Incubation of THP-1 macrophages with oxLDL *versus* nLDL induced: (a) intracellular accumulation of free and esterified 7-KC; (b) ER stress, by the activation of eukaryotic initiation factor-2 α (eIF2α) and by the up-regulation of C/EBP homologous protein (CHOP); (c) increase of NADPH oxidase activity and ROS production; (d) increased CRP gene expression and protein secretion, and (e) increased MMP-9 gene expression, protein secretion and enzymatic activity. Furthermore, the experiments demonstrated that oxLDL-induced CRP gene expression and protein secretion were reduced by the inhibitors of oxidative stress, while oxLDL-induced MMP-9 gene expression, protein secretion and enzymatic activity were diminished by the inhibition of the ER or oxidative stress. Oxidized LDL have notable effects on human macrophages: they induce CRP secretion *via* a mechanism that involves the oxidative stress, and enhance and modulate MMP-9 secretion and enzymatic activity by the activation of both ER and oxidative stress.

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NONALCOHOLIC FATTY LIVER DISEASE: A NEW CARDIOVASCULAR RISK FACTOR?

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Nonalcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in developed countries, with an incidence of 30-35% in the general population. Nonalcoholic fatty liver disease is not only associated with liver-related morbidity and mortality, but also with an increased risk of cardiovascular disease (CVD), abnormalities of cardiac function and structure, valvular heart disease and arrhythmias. Moreover, there is a large body of clinical and epidemiological evidence supporting the concept that NAFLD is strongly associated with major cardiovascular risk factors, including type 2 diabetes mellitus, obesity, dyslipidemia, hypertension and insulin resistance. The mechanisms linking NAFLD with CVD are not fully understood yet, but recent studies show that NAFLD, especially in its more severe forms, exacerbates systemic/hepatic insulin resistance, causes atherogenic dyslipidemia and releases a variety of pro-inflammatory, pro-coagulant and pro-fibrogenic mediators that may play important roles in the pathophysiology of cardiac complications. These findings suggest that the clinical impact of NAFLD on CVD risk deserves particular attention and patients with NAFLD may benefit from more intensive surveillance and early treatment to decrease the risk of CVD and other cardiac complications. Although the mechanisms underlying the association between NAFLD and CVD are not fully understood and the prognostic value of NAFDL in CVD risk stratification has yet to be determined, attention must be paid to this association, given that many patients with NAFLD, especially those with nonalcoholic steatohepatitis (NASH) will develop major CVD events and die prior to the development of advanced liver disease. In conclusion, far from being a benign and "paraphysiological" condition, NAFLD should be viewed as a complex and multi-faceted disease often calling for multi-disciplinary intervention, a collaboration between cardiologists and hepatologists being essential for the treatment and management of these patients.

KIDNEY IN NONALCOHOLIC FATTY LIVER DISEASE

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Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries that is predicted to become also the most frequent indication for liver transplantation by 2030. The term „nonalcoholic fatty liver disease" was coined by gastroenterologists almost 20 years ago to define a spectrum of progressive liver disease that encompasses simple steatosis and nonalcoholic steatohepatitis (NASH), which is characterized by the presence of steatosis, necroinflammation, and/or fibrosis, and ultimately cirrhosis. The same entity was also well known to diabetologists and regarded as an epiphenomenon of the metabolic syndrome. In recent years, this perspective has completely been changed by the combined research efforts of both gastroenterologists and endocrinologists, determining that NAFLD itself can lead to an increased risk of developing metabolic syndrome and its complications beyond established predictors. The primary focus of this review will be on the association between NAFLD and a spectrum of extrahepatic diseases that were traditionally linked to metabolic syndrome such as type 2 diabetes mellitus, cardiovascular disease, chronic kidney disease, and cancers. The top three leading causes of death in patients with NAFLD in descending order are cardiovascular disease, cancer, and liver disease. It is clear now that the increased risk of metabolic and macro- and microvascular complications in NAFLD stems from the associated features of metabolic syndrome. However, NAFLD itself may contribute to the spectrum of risk factors associated with insulin resistance. Hepatic lipid accumulation in NAFLD impairs hepatic glucose and lipid metabolism further increasing the risk of type 2 diabetes mellitus and of cardiovascular disease, independently of established risk factors. The incidence, prevalence, and severity of these complications are proportional to the histological severity of liver damage suggesting that NAFLD, but particularly NASH, can also contribute to the low-grade inflammatory state through the systemic release of several markers of inflammation, oxidative stress, and of procoagulant factors. The clinical implication of these findings is that patients with NAFLD require a multidisciplinary evaluation, with a major focus on type 2 diabetes mellitus and cardiovascular disease complications and may benefit from more intensive surveillance and early treatment interventions to decrease the risk for cardiovascular and kidney complications. The possible link between NAFLD and chronic kidney disease (CKD) has recently attracted considerable scientific interest. Several large cross-sectional population and hospital-based studies, involving both adults without diabetes and patients with diabetes, have shown that the prevalence of CKD (defined as either decreased estimated glomerular filtration rate (GFR) and/or overt proteinuria) is increased in people with NAFLD. These studies have used either ultrasonography or biopsy to diagnose NAFLD and have excluded patients with end-stage renal disease, cirrhosis and those with known causes of chronic liver disease (alcohol abuse, viral hepatitis and use of hepatotoxic drugs in all studies and also hemochromatosis and autoimmune hepatitis in some studies). In these studies, the prevalence of CKD in patients with NAFLD ranged from approximately 20–55% compared to 5–35% in patients without NAFLD. Importantly, most of these studies, including those that used liver biopsy to diagnose NAFLD, reported that the presence and severity of NAFLD was associated with CKD stages, independently of established cardio-renal risk factors. To date, there is a paucity of published data regarding the risk of developing CKD in patients with NAFLD. Notwithstanding these limitations, the published prospective studies have consistently reported an independent association between NAFLD and increased risk of incident CKD with HRs for CKD that ranged from approximately 1.3–1.9. Very recently, in a well-conducted systematic review and meta-analysis (63,902 participants, 20 cross-sectional and 13 longitudinal studies included), Musso *et al* (1) confirmed that NAFLD as diagnosed by histology, imaging or liver enzyme elevation was significantly associated with an increased risk of prevalent (OR 2.12, 95% CI 1.69–2.66) and incident CKD (HR 1.79, 95% CI 1.65–1.95). Additionally, NASH was associated with a higher prevalence (OR 2.53, 95% CI 1.58–4.05) and incidence (HR 2.12, 95% CI 1.42–3.17) of CKD than simple steatosis. However, fur-

ther longer prospective studies in larger cohorts of patients with biopsy-proven NAFLD are needed to confirm these findings, and to determine whether improvement in NAFLD (or future treatments for NAFLD) ultimately will prevent or delay the development and progression of CKD. Moreover, because CKD has many potential causes, it also will be of great interest to characterize the renal injury manifestations associated with NAFLD and clarify, in the future, whether NAFLD may selectively contribute to the pathogenesis of different types of kidney disease (1-3).

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NEW TOOLS FOR PREDICTION AND ASSESSMENT OF OBESITY IN CHILDREN

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Obesity in children became a public health problem. The role of adipose tissue is not only to store and metabolize lipids, but to function as an endocrine organ by releasing adipokines involved in the regulation of inflammation (e.g. interleukins, leptin, adiponectin), coagulation (plasminogen activator inhibitor-1, PAI-1), feeding behavior (leptin) and insulin resistance (TNF-α). The adipokine levels rise with an increase in adipose tissue and adipocyte volume and can affect the health on long term. Therefore, it was important to develop new tools for assessing these substances as well as of other ones like E2, T2, DHT and bisphenol A in childrens' saliva and whole blood. Stochastic sensors based on carbon and textile matrices and nanostructured materials were proposed as new tools for the screening of saliva and whole blood. No sampling was needed for either saliva or whole blood for detection and quantification of E2, T2, DHT, bisphenol A, TNF-α, IL-6, MCP-1, PAI-1, and leptin. The sensors were introduced in 30-50μL of sample, and these markers were identified accordingly with their signature from the diagrams. After their identification, they were quantified using the calibration equations. The new tools designed based on stochastic sensors were able to detect and quantify E2, T2, DHT, bisphenol A, TNF-α, IL-6, MCP-1, PAI-1, and leptin, at levels between fg/mL to ng/mL with high sensitivity and selectivity. The range of utilization made possible the analysis of these substances in children's saliva and whole blood, favorizing the prediction and assessment of the obesity in children, but also identifying the risk factors for associated illnesses. The new tools can detect fast and reliable the hormones, bisphenol A, citokines and adipokines in saliva and whole blood samples of children, being good alternatives for accredited methods like ELISA. For the assay of many of these substances, there is no alternative in clinical analysis for samples like, e.g., saliva samples, due to the low levels of these substances in the biological fluids of children.

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INCREASED METABOLIC RESPONSE OF SUBCUTANEOUS ADIPOSE TISSUE-DERIVED STROMAL CELLS AFTER *IN VITRO* EXPOSURE TO SERUM FROM OBESE SUBJECTS

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Subcutaneous adipose tissue is an important source of multipotent stem cells and a key player for the pathophysiology of obesity. These two features of adipose tissue are interconnected, and despite of well-recognized beneficial outcome of stem cells in regenerative therapies, their involvement in adipose tissue dysfunction in obesity and related metabolic disorders has not been entirely uncovered. The aim of this study was to investigate the proliferation, differentiation and the metabolic response of subcutaneous adipose-derived stromal cells (ADSC) after simulation *in vitro* of the obesity conditions. Human ADSCs have been isolated from subcutaneous adipose tissue of non-obese subjects and maintained in culture until the 5th passage. The cells were cultured in the presence of sera from obese (oADSC) and non-obese patients (cADSC) followed by examination of their proliferation and differentiation into an adipose lineage. After 21 days of differentiation, we evaluated the intracellular lipids accumulation (by Oil-Red O staining), the gene expression of the adipogenic markers, such as PPARγ2, C/EBPα, FABP4, and LPL (by Real Time-PCR), the levels of generated ROS (using the fluorescent dye DCFH-DA), the activation of eIF2α and IRE1α - related metabolic stress pathways, and the expression of pro-inflammatory proteins MCP1 and E-selectin (by Western blotting). The results showed that, compared to cADSC, oADSC exhibited: (*i*) a slower process of differentiation, (*ii*) a hyperplasic phenotype, (*iii*) accumulation of smaller lipid droplets inside the cytoplasm (indicative for altered metabolic adaptation), (*iv*) enhanced expression of adipogenic marker genes of PPARg2, C/EBPa, FABP4, and LPL, (*v*) stimulation of ROS production and enhanced protein expression of metabolic stress markers, such as eIF2α and IRE1α, and (*vi*) installment of a pro-inflammatory condition associated with augmented protein expression of MCP1 and E-selectin. Based on the above *in vitro* results one can infer that exposure of stromal cells from subcutaneous adipose tissue to conditions similar to those in obesity induced a switch from their basic lipid storage function to a pro-inflammatory phenotype and this change may contribute to adipose tissue dysfunction in obesity.

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DYSFUNCTIONAL HDL IN THE SMALL INTESTINE OF HYPERLIPIDEMIC HAMSTERS IS GENERATED BY THE INCREASED ENDOPLASMIC RETICULUM STRESS - REVERSE EFFECT OF LOWERING INTESTINAL LIPID TRANSPORT BY PROBIOTICS

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Dyslipidemia is one of the causes of obesity, diabetes and atherosclerosis. Decreased HDL-cholesterol is characteristic for dyslipidemia. The new hypothesis of "HDL functionality" states the relevance of HDL functionality over HDL-cholesterol for the risk of developing cardiovascular diseases. HDL proteins are synthesized in the liver and the small intestine. Paraoxonase1 (PON1), the main antioxidant enzyme associated with HDL, is known to be synthesized in the liver, and its decreased activity is connected with HDL dysfunction. There is a debate whether PON1 synthesis takes place in the intestine. We aimed to investigate PON1 synthesis in the small intestine of hamsters and the mechanisms linking hyperlipidemia (HL) with the dysfunctional HDL and the association with the aortic valves lesions. We used male Golden Syrian hamsters fed either standard chow (N), HL diet (3% cholesterol and 15% butter) for 20 weeks, or HL diet supplemented with probiotics (*Lactobacillus acidophilus* and *Bifidobacterium animalis*) (HLP) for the last 4 weeks of the experiment. The expression of PON1, apolipoprotein AI (apoAI), lipid transporters (Niemann Pick C1 like 1 - NPC1L1, microsomal triglycerides transfer protein – MTTP), the endoplasmic reticulum stress (ERS) sensors, the transcription regulators, and the oxidized lipids were assessed in the small intestine, the liver and serum. The lesion areas of the aortic valves were evaluated. All animals fed the fat diet developed HL, and 50% HL and hyperglycemia (HLHG) after 16 weeks. PON1 and apoAI expression were decreased in the small intestine of HLHG< HL< N hamsters. In parallel, ERS was activated, oxidized lipids levels and lipid transporters NPC1L1 and MTTP expression increased, along with the decrease of liver X receptors (LXR) and peroxisome proliferator-activated receptorγ (PPARγ). In the liver, the expression of PON1 was reduced, the expression of apoAI, ERS sensors and oxidized lipids levels increased, along with the decrease of LXR and PPARγ. PON1 protein and activity decreased in HLHG<H<N sera, along with the increase of oxidized LDL and the lesion areas of the aortic valves (HLHG>HL). The administered probiotics diminished NPC1L1 and MTTP expression, and increased PON1, apoAI, LXR and PPARγ expression in the small intestine, in parallel with the decreased lesion areas of the aortic valves (HLHG>HL). The HL diet induces the increase of the lipid transporters, ERS and oxidative stress, along with the reduction of PON1, apoAI, LXR and PPARγ expression in the small intestine. The excess oxidized lipids, the reduced PON1 levels and activity in sera are indicative of the presence of dysfunctional HDL and are accompanied by the development of aortic valves lesions. Diminution of the intestinal cholesterol uptake after probiotics administration restored PON1 expression and activity, upregulated LXR and PPARγ, and reduced the aortic valves lesion areas.

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A CORRELATION BETWEEN INTERMEDIARY DIABETES MELLITUS AND OBESITY

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Diabetes mellitus (DM) and obesity have an interwoven relationship. Type 2 diabetes mellitus (T2DM) is strongly associated with obesity which stands out as a risk factor. Besides obesity, risk factors for T2DM include physical inactivity and family history of metabolic disorders and/or atherosclerosis. All of the above risk factors should indicate a more narrow range of research involving a refined and complex process, namely the gene expression patterns. In order to better understand the diabetogenic mechanisms, numerous gene expression studies have been conducted for unraveling the relationship between these heterogeneous phenotypes. However, diabetes studies related to the main effectors of gene expression (the promoters of genes) were rarely conducted. Here we intend to present the relationship between these effectors in more detail in the context of obesity and diabetes. In our study we used a new method of analysis known as „DNA pattern" method, which has been used in the past to analyze the structural properties of promoters of genes in various species. Among the analyzed promoters of genes associated with T1DM, we mention the PTPN22, TLR7, CTLA4, GSDMB, STAT4, IL7R, C1QTNF6, CD55, CTSH, ERBB3, HLA-DQA1, HLA-DQB1, HLA-DRB1, HLA-DPB1 and INS gene promoters (Fig. A) and for T2D the CAMK1D, DUSP9, HHEX, IRS1, MADD, NOTCH2, TP53INP1, VPS13C, WFS1, ZFAND6, HMGA2, PPARG, CDKN2AIP, PROX1 and TCF7L2 gene promoters (Fig. B). In connection with obesity, we considered the promoters of the following genes: FTO, PCSK1, MTCH2, SH2B1, NEGR1, GNPDA2, INSIG2, TMEM18, KCTD15, BDNF. The „DNA pattern" methodology allows the determination of the coexpression relationships between genes. Thus, the overlapping positions (or close positions) of promoters of these genes inside the distribution indicate that those promoters use transcription factors in common. A key goal for treatment and prevention of T2DM is the reduction of obesity. The main treatment of both conditions is increased physical activity and reduced caloric intake. Thus, by modifying these environmental factors, a direct modification of gene expression patterns is produced. As can be seen in figure below, the promoters of genes associated with the two main phenotypes (T1DM and T2DM) of diabetes contain different DNA patterns, indicating different mechanisms of action. However, the intermediary diabetes mellitus (IDM) and obesity phenotypes show forms of intermediate promoter classes, which makes their associated genes functionally receptive to transcription factors specific to both T1DM and T2DM phenotypes. Accordingly, the close distribution of promoters of genes associated with the analyzed phenotypes depicts a functional correlation (co-expression) of these genes. The figure below shows the general distribution of the four phenotypes and indicates the promoters of genes positioned at the extremes of the distribution. The individual distribution of promoters of genes associated with T1DM, T2DM, IDM and obesity it is shown in Figure A whereas the global distribution (the mean values) of the four phenotypes are shown in Figure B. Our distribution suggests that there is a strong correlation between IDM and Obesity (Fig. B). The unexpected location of gene promoters associated with obesity, closer to T1DM (Fig. B), could indicate the immune response known as low grade inflammation commonly associated with obesity. In our study we used the promoters of genes associated with T1DM, T2DM, IDM and obesity. Our analysis showed that IDM and obesity genes are equipped with promoters prone to common triggers. Also, IDM and obesity associated genes (with promoters similar in structure) are functionally receptive to transcription factors specific to both T1DM and T2DM phenotypes.

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