International Journal of Obesity (2007) 31, S46–S77
 0307-0565/07 $30.00
www.nature.com/ijo

POSTER SESSION: TRACK 1 - CELLULAR AND MOLECULAR MECHANISMS

T1:PO.01

Obesity and hyperlipoprotheinemia in assessing of the severity of coronary artery disease

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The relationship between obesity, hyperlipoprotheinemy and the form of severity of coronary artery disease, were analised in the patients on Clinic for Cardiovascular diseases University Clinical Center Tuzla. The goal of the research was to determine does obesity with hyperlipoprotheinemy couses the worst form of coronary artery disease.
Materials and methods: There were 140 patients (57,88 ± 9,33 years); 77 men and 63 women in randomised study during six months. The patients were divided in two groups: a) experimental group: the patients who were obese by central type of obesity (waist circumference a bove 88 cm in women and 102 cm in men,and b) control group the patients who were not obese by central type of obesity. BMI, WHR ratio, waist circumference, cholesterol, LDL, HDL, tryglicerides were measured in all patients. Coronarography with onewessels disease were easier form of disease, and two and treewessels disease were the worst form of disease.
Results: The average waist circumference was 94, 02 ± 4,72 cm. Average BMI was 27,40 ± 5,01, Between all patients, 59 of patients had the worst form of disease, and 81 of the patients had easier form of disease. Statistically important difference were found (P=0,0008, OR=2,84). Between 59 of the patients with the worst for m of disease, 19 of the pati ents had BMI>30, and between 81 patients with easier form of disease 12 of the patients had BMI>30 (P=0,05, OR=2,39).Statistically important difference found in the level of cholesterol (P=0,0012, OR=2,38), the level of LDL (P=0,02, OR=2,38), and HDL cholesterol (P=0,01, OR=2,72). The difference in WHR ratio were statistically important, too (P=0,002).
Conclusions: The results of the research showed that pathological level of the LDL cholesterol in the serum of the patients joined with pathological levels of the waist circumference and BMI were independent predictors of appearance of the worst form of coronary artery disease of the hart (OR=3,9).

T1:PO.02

Interleukin-6 -572 G/C Polymorphism in relation to Metabolic Syndrome Components among Young Adolescents in Taiwan

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To evaluate IL-6 -572G/C promoter polymorphism (rs1800796) in relation to metabolic syndrome (MS) components among young adolescents in Taiwan. After multi-stage sampling, we enrolled 934 school children (453 boys and 481 girls) in Taipei at 2003. Modified NCEP ATP-III criteria were applied to define MS (with age- and gender-specific 90th percentile cut-off point of study variables). Subjects have three or more of following cardio-metabolic abnormalities considered as MS: high blood pressure (BP), high fasting glucose, high triglyceride (TG), low HDL-C and obesity. The genotype of IL-6 -572G/C SNP was determined by Pre-design TaqMan® assay using TaqMan® probes and Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The frequency of IL-6 -572 promoter polymorphism CC, CG and GG was 58.0, 36.9 and 5.1% for boys and 63.2, 31.6 and 5.2% for girls. IL-6 -572 G/C polymorphism was associated with BP and TG levels among children. Boys with (CC+CG) genotype had higher systolic BP (SBP) and TG than (GG) genotype (P<0.05). The CG genotype boys had higher TG level than GG genotype (71.8 ± 33.1 vs. 55.1 ± 22.1, P<0.05). However, the girls with C-allele carriers had higher TG level and more percentage of high glucose level than G-allele carriers (P<0.05). The odds ratio of high glucose level for C-allele carriers girls was 1.54 (95% CI: 1.00~2.33) when compared with G-allele carriers. IL-6 -572 G/C polymorphism is associated with blood pressure, TG and glucose levels in children.

T1:PO.03

Glycemic control between private and university hospital

Qari Faiza[1]

[1]Kauuh

Objective: Determine and compare a good target level of blood sugar control assessed by HBAIc, blood pressure and serum LDL – cholesterol in diabetic patients attending out -patients clinics at KAUH and patients attending private hospital Saudi Arabia.
Methods: Across section study conducted at two month period between January 2005 and February 2005 in two centers (KAUH) and Erfan hospital.
Results: Two hundred patients, one hundred from each hospital were enrolled in the study. Females a ccoun ted for 70% at KAUH group versus 54 % i n Erfan group. Saudi patients attending Erfan group were 62 % compared to 51 % in KAUH group. Mean HbAIc was almost the same in both groups 7.8+/ -1.8 mmol/L. good and acceptable HBAIc was observed in 58 % at KAUH group versus 54 % at Erfan group. The blood pressure target control was good in both groups; however target LDL – cholesterol was scientifically better in Erfan group 1.88+/ -1.2 versus 3.22+/.9 m mol/ L in KAUH group with significant p value of 0.0001.
Conclusions: Even after great effort s, a target level of HBAIc glycated hemoglobin not achieved in both groups of patients – in private and university hospitals. LDLcholesterol was not achieved in university hospital, where as low rate of aspirin use was not achieved in both groups. Efforts are needed to improve compliance to diet and drug regimens and to identify and treat risk factors in each patient with the aim to reach target recommendations for Hebraic, blood pressure and LDL -cholesterol.

T1:PO.04

Glycemic control between university and private hospital

Qari Faiza[1]

[1]Kauuh

Objective: The aim of the study is to determine the association of fatty liver diagnosed by ultrasound and obesity in patients presented to King Abdul aziz university hospital.
Methods: A clinical notes review was performed of all patients undergoing evaluation for fatty liver associated with obesity over one year period between April 2003-to April 2004. Data included age, gender, nationality, BMI, serum level of alanine (ALT) and aspartate (AST) transaminases, biluru bin, albumin, HbA1 C, cholesterol, triglyceride, LDL, and TSH, and clinical presentation of abdominal pain or the presence of hepatomegaly.
Results: A total of 235 subjects were enrolled in the study .The mean age of the study group was 46 +/ -14.4 years with 82 males (35 %) and 153 females (65%). Mean +/- SD aspartate aminotransferase level was 43.9+/6.18.1units /L, alanine aminotrnsferease was 36.2+/ -5.1 units /L. Values of transaminase above the normal range was present in 15 (6.4%) patients only. Whereas values of cholesterol and triglyceride above normal range was seen in (7.2%) 17 patients. Over weight and obesity were the main risk factors in our study group. Mean BMI was 33.6 +/ - 7.5 Kg/m^2. Obesity with diabetes is the most important risk for fatty liver. 78 (33 %) of our study group patient were diabetic. Other risk factors associated with fatty liver are metabolic syndrome which is reported in 14.9% and hypothyroidism in 3.8 % subjects.
Conclusion and recommendations: Overweigh and obesity is the most important risk fact or for fatty liver in Saudi Arabia. It is more prevalent in females. Ultrasound appears to be a useful non-invasive tool to determine liver involvement with fatty liver in obese adult even in the absence of hypertraninasemia. We should encourage obese subjects for gradual weight reduction to improve the liver abnormalities.

T1:PO.05

Circulating nerve growth factor levels are increased in obesity and metabolic syndrome

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Introduction: Nerve growth factor (NGF) could be involved in the development and progression of inflammatory and immune disease playing an important role in the ethiopathogenic mechanisms underlaying obesity and the metabolic syndrome (MetSyn)
Methods: Neurotrophins plasma levels were evaluated in 146 adult women (BMI 20-70 kg/m2) with or without metabolic syndrome. Subcutaneous adipose tissue samples were also obtained in a subgroup of morbid obese and normal-weight females where NGF expression was analyzed by real-time PCR.
Results: NGF plasma levels were 1,4-fold higher in obese compared with normoweight subjects. Plasma NGF was, however, lower in a group of morbidly obese subjects than in obesity, but it remained elevated relative to the normoweight group. Plasma NGF was significantly correlated with BMI (r=0.23; P=0.03), percentage body fat (r=0.22, P=0.04) and waist circumference (r=0.29; P=0.01) in non morbidly obese subjects. NGF was positively related to inflammatory markers such as plasma leptin levels and sTNFR1.
NT3 and BDNF were more related to lipid profile markers than to BMI, adipose tissue distribution or peripheral inflammatory markers. NGF mRNA levels were increased in a subgroup of morbidly obese compared with the normoweight subjects. Subjects with type-2-diabetes, abdominal fat distribution or the MetSynd showed significantly higher levels of NGF. MetSynd was the only independent predictor of the variability observed in the NGF plasma values.
Conclusion: NGF is upregulated in obesity, type-2-diabetes and the MetSynd. Whether this neurotrophin may contribute to inflammation and the metabolic derangements associated to obesity remains to be elucidated.

T1:PO.06

Plasma adiponectin distribution in a mediterranean population and its association with cardiovascular risk factors and metabolic syndrome

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Objective: To evaluate the distribution of adiponectin in a Mediterranean adult population and its relationship with cardiovascular risk factors and metabolic syndrome.
Design and Methods: Cross-sectional study performed in a representative sample of 1023 subjects from a Spanish Mediterranean population. Metabolic syndrome was defined using the diagnostic criteria of the Adult Treatment Panel III. Anthropometrical parameters were measured and biochemical analyses were performed in fasting conditions. Plasma insulin levels were measured and HOMA IR was calculated. Plasma adiponectin levels were measured by ELISA.
Results: Plasma adiponectin levels were significantly higher in women than in men after adjusting for differences in BMI (11.20 ±0.18 vs 7.52 ±0.27 respectively, P<0.001). Significantly lower levels of adiponectin were also observed in women with obesity, abdominal obesity, hyperglycaemia or diabetes, low HDL cholesterol or hypertriglyceridemia. Otherwise, of the men, only those with obesity, low HDL cholesterol or hypertriglyceridemia showed significantly lower plasma levels of adiponectin. Plasma adiponectin levels were lower in patients with metabolic syndrome both in women (11.69 ±4.78 vs 9.57 ±4.132, P<0.001), and in men (7.81 ±3.88 vs 6.09 ±2.88, P<0.001 absence or presence, respectively). In a multiple regression analysis, gender, waist circumference, serum C-reactive protein serum levels and HOMA IR explained 20% of its variability.
Conclusions: Adiponectin plasma levels are more closely related to the components of the metabolic syndrome in women than in men in a Mediterranean population. The results of this study support the hypothesis that adiponectin may play a role in the development of diabetes and cardiovascular disease.

T1:PO.07

New lychee-derived polyphenol Oligonol converted into a low-molecular form reduces oxidative stress in adipocytes

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Recently, it has been strongly suggested that increased oxidative stress in adipocytes is one of causes of obesity-associated metabolic syndrome. In this study, we investigated the antioxidative effect of new lychee-derived polyphenol Oligonol converted into a low-molecular form (Amino Up Chemical Co., Ltd., Sapporo, Japan) in adipocytes. Six-week-old male C57BL/6J mice were divided randomly into four groups: 1) C mice: control mice; 2) HFD mice: mice given a high fat diet for 5 weeks; 3, 4) HFD + Oligonol or LP mice: mice given HFD with Oligonol or with ordinary lychee-derived polyphenol (not a low-molecular form) (100 mg/kg each). Oxidative stress in epididymal white adipose tissues (WAT) was measured by thiobarbituric acid reactive substance (TBARS). Although the levels of WAT mass and TBARS in HFD mice were significantly higher than those of C mice, Oligonol definitely inhibited such HFD-induced increases. Expression of TNF-α and•MCP-1 genes in WAT of HFD mice was significantly higher than that in C and HFD + Oligonol mice, while the expression of adiponectin gene was suppressed in HFD mice, but not in HFD + Oligonol mice. Moreover, the expression of PAI-1 gene in HFD + Oligonol mice was less than that in HFD mice. On the other hand, the expression of EC-SOD gene was enhanced only in WAT of HFD + Oligonol mice. These results suggest that the new polyphenol Oligonol used has antioxidative effects and attenuates an increase in the expression of genes for metabolic syndrome-related adipocytokines in WAT of HFD mice.

T1:PO.08

Angiotensin-converting enzyme gene insertion/deletion (I/D) polymorphism diminishes autonomic nervous system activity in young healthy Japanese females

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Introduction: An insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene reportedly has effect on abdominal adiposity. Reduced ANS activity has also been reported to be involved in the development of obesity, however, a link between this polymorphism and autonomic function is still uncertain. We therefore investigated whether or not ACE I/D polymorphism affected the ANS activity in young healthy Japanese females.
Subjects and methods: One hundred and one subjects (20 –25yrs) were genotyped for I/D polymorphism of the ACE gene by applying a PCR -restriction fragment length polymorphism using buccal samples. The ANS activity was assessed by power spectral analysis of heart rate variability. Energy intake and physical activity level were evaluated using 24h-recall method and lifestyle questionnaires.
Results: The frequency of the II, ID and DD genotypes was 0.35, 0.54 and 0.11, respectively. No group difference was found in anthropometric indexes, blood pressure, energy intake, and physical activity level between the II+ID and DD groups. As to ANS activity, a very-low frequency power reflecting sympathetic nervous system (SNS) activity related to energy metabolism regulation, a low frequency power jointly regulated by both SNS and parasympathetic nervous system (PNS) activities, and a high frequency power that sorely reflected PNS activity were all significantly lower in the DD group than those in the II+ID group.
Conclusion: The present results suggest that the ACE I/D polymorphism diminishes the ANS activity in young females. These findings raise the possibility that ACE I/D polymorphism may be one of genetic marker s for future pathogenesis of obesity.

T1:PO.09

Association between liver X receptor alpha gene polymorphisms and metabolic syndrome in French populations

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The metabolic syndrome is a complex and multifactorial disorder often associated with type 2 diabetes and cardiovascular diseases. The liver X receptor alpha (LXRA) plays numerous roles in metabolic pathways involved in metabolic syndrome. In the search for susceptibility genes to metabolic syndrome, we hypothesized that common genetic variation in LXRA gene influences metabolic syndrome susceptibility. Two large French population - based studies (n=1130 and n=1166) including overall 664 individuals with and 1626 individuals without metabolic syndrome were genotyped for three polymorphisms, accounting for >96% of the genetic variability of *LXRA*. We found that the rare allele of one of the polymorphisms was consistently associated with a 30% reduction in risk of metabolic syndrome in the two independent population samples (adjusted OR [95% CI]=0.68 [0.53–0.86], P=0.001 in the combined sample). Moreover, it was associated with an increase in plasma HDL-cholesterol concentrations. These results suggest that LXRA plays an important role in the genetic susceptibility to metabolic syndrome, partly explained by its action on HDL metabolism. 1. Conflict of Interest: None Disclosed. 2. Funding. Research relating to this abstract was funded by the Conseil Régional du Nord-Pas de Calais, the CPAM de Sélestat, the Association Régionale de Cardiologie d'Alsace, ONIVINS, Parke -Davis Laboratory, the MGEN, the Réseau Nati onal de Santé Publique, the Direction Générale de la Santé, the INSERM, the Institut Pasteur de Lille, and the Unité d'Evaluation du CHU de Lille. This work was also supported by CRESCENDO, an Integrated Project funding from FP6 (contract n° LSHM - CT-2005- 018652).

T1:PO.10

Search for adipose tissue mRNA biomarkers of changes in dietary content of energy restricted diets in obese humans

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The outcome of hypocaloric diets is highly variable at the individual level. The European programs, NUGENOB and DioGenes focus on gene-nutrient interactions during nutritional weight reducing challenges in obese subjects. The present study aims to characterize biomarkers of dietary intervention using transcriptomic analyses of human adipose tissue(AT). 2 groups of 24 obese women following energy restricted diets with moderate-fat or low fat content for 10 weeks have been selected from a cohort of ~750 obese subjects. The groups are similar regarding changes in weight and metabolic parameters during the diets. Probes made from the subcutaneous AT RNA both before and after dietary restriction period were hybridized to pangenomic microarrays. Different strategies were used to analyze the data. The search for calorie restriction sensitive genes was performed using SAM, a permutation algorithm with multiple testing adjustments which yielded 1800 differential transcripts, the large majority being down regulated as shown on a smaller subset of genes (Viguerie, Diabetologia, 2005). In order to set up a list of transcripts which changes in expression were different regarding the nutrient composition of the hypocaloric diet, two independent statistical strategies have been tested: PAM, a partitional clustering algorithm and random forest, a training-validation approach. The lists from each method were merged and genes were classified. The 10 top ranking transcripts have been tested using RT-qPCR on an enlarged set of subjects. Investigation of the biological relevance of each putative biomarker will now help to connect energy metabolism, nutrients and gene expression changes under caloric restriction.

T1:PO.11

Differential regulation of adipose tissue inflammation-related genes during very low calorie diet and weight maintenance phase in human obese subjects

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Adipose tissue secretes inflammation-related molecules that may be involved in the modulation of insulin sensitivity. Caloric restriction programs have been shown to improve the adipose tissue inflammatory status of obese subjects. The present work aimed at determining the relationship between the expression profiles of inflammation-related genes and the different phases of a weight loss program. Eight obese women followed 4 weeks of very low calorie diet (VLCD) and 5 to 8 months of a weight maintenance program (WM). Transcriptomic analysis of subcutaneous adipose tissues obtained at baseline, after the VLCD and after WM was performed using pangenomic oligonucleotide microarrays. Among significantly regulated genes, we observed a marked regulation of genes involved in immunity and defense processes in the different phases of the protocol. Variations of the expression levels of these genes were analyzed concomitantly with changes in anthropometric and insulin sensitivity parameters. Insulin sensitivity was improved during VLCD and remained ameliorated after WM. A fraction of inflammatory-related genes were downregulated during VLCD and tended to return to baseline levels during WM. On the contrary, WM regulated the expression of genes which remained unaffected by VLCD. Regulation of these genes could contribute to the long term effect of weight loss on insulin sensitivity. This kinetic analysis during a weight loss program reveals that inflammation-related genes are differentially regulated by the energy deficit during severe calorie restriction and the new energetic status obtained during a weight maintenance phase. It may help to delineate new biomarkers of insulin resistance in human obesity.

T1:PO.12

Microarray data of human white adipocytes overexpressing PGC-1alpha and regulation of glycerol kinase by PPARalpha

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Plasma free fatty acids released from white adipose tissue may contribute to the metabolic abnormalities found in obese subjects. Expression of the transcriptional coactivator peroxisome proliferator -activated receptor gamma (PPARgamma) coactivator 1alpha (PGC-1alpha) in human adipocytes leads to a PPARgamma - dependent induction of the uncoupling protein UCP1 and promotes fat oxidation. Here, pangenomic microarray experiments were performed to get an exhaustive view at changes in gene expression induced by PGC-1alpha. Among the large number of genes regulated by PGC-1alpha independently of PPAR gamma, were new targets involved in metabolism including the gene encoding glycerol kinase (GyK). The induction of GyK by PGC- 1alpha was observed at the levels of mRNA and enzymatic activity. PPARalpha was also upregulated by PGC -1alpha. Its activation led to an increase in GyK expression and activity. PPARalpha was shown to bind and activate the GyK promoter in PGC -1alpha expressing human adipocytes. In vivo data in different mouse models confirmed the role of PGC-1alpha and PPARalpha in the regulation of GyK. The induction of GyK by PGC-1alpha and PPARalpha offers a new strategy to promote fat utilization in fat cells through the generation of a futile cycle between triglyceride hydrolysis and fatty acid reesterification. Moreover, this work uncovers novel pathways regulated by PGC -1alpha and reveals that PPAR alpha controls gene expression in human white adipocytes.

T1:PO.13

The first intron of the human UCP3 gene governs expression in skeletal muscles *in vivo*

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Uncoupling protein-3 (UCP3) is an inner mitochondrial membrane transporter mainly expressed in skeletal muscles in humans and in brown adipose tissue and skeletal muscles in rodents. UCP3 may protect against lipid induced oxidative damages and play a role in fatty acid oxidation. In humans, UCP3 content is higher in fast contracting glycolytic muscles than in slow contracting oxidative muscles. Here, we have studied the molecular mechanisms determining UCP3 expression and characterized the sequences responsible for specific muscular expression. We have created transgenic mice bearing a 16 kb sequence of the human UCP3 gene (hUCP3) including the promoter and all intron-exon sequences. The transgene expression was comparable to that of the endogenous murine UCP3 gene (mUCP3). Further transgenesis experiments with a hUCP3 promoter chloramphenicol acetyl transferase (CAT) gene construct did not result in expression of the reporter gene in mouse tissues. Creation of additional lines with intron 1 linked to the promoter reporter gene construct resulted in an expression profile of the CAT gene in skeletal muscles comparable to that of hUCP3, i.e., higher expression in glycolytic than in oxidative muscles. For instance within intron 1, the 5' 600 bp-long region is the smallest fragment conferring skeletal muscle expression of the transgene. Therefore, hUCP3 expression in skeletal muscles is not solely conferred by the promoter but depends on *cis* acting elements in intron 1. Studies with cellular models revealed several important regulatory elements in the hUCP3 promoter; however our transgenesis experiments demonstrate the necessity of intron 1 *in vivo*.

T1:PO.14

The Leu7Pro7 genotype of preproNPY leads to rapid increase in intima media thickness and decreasing vasodilatation responsiveness in over-weight patients with diabetes -- two-year preliminary results

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Neuropeptide Y (NPY) is a sympathetic transmitter that has various physiological functions. Leucine7 to Proline7 (Leu7Pro) polymorphism of prepro*NPY* is associated with altered sympathetic transmitter concentrations and vascular diseases. Middle-aged men with Leu7Pro7 genotype have enhanced endothelium-dependent flow-mediated endothelial dilatation (FMD). We have monitored the progression of brachial FMD and brachial artery intima-media thickness (bIMT) in patients with type 2 diabetes (T2D). Twelve patients with T2D without diabetic complications having Leu7Pro7 genotype and 19 with Leu7Leu7 genotype attended an annual follow-up including a non-invasive ultrasound assessment of vascular function and structure. To measure FMD, ultrasound scans were taken at rest and after reactive hyperemia induced by a forearm cuff-release. Sublingual glyceryl trinitrate spray (1.2mg) was used to investigate endothelium independent nitrate dependent vasodilatation (NMD). The measurements were repeated after a 2 year follow-up. *P*-values were obtained by t-test for continuous and Mann-Whitney for skewed variables. Baseline brachial vessel size, FMD, NMD and bIMT were similar between Leu7Pro7 and Leu7Leu7 genotypes. The 2-year increase in bIMT was significantly greater in the Leu7Pro7 genotype than in the Leu7Leu7 genotype (0.07±0.06 vs. 0.03±0.08 mm, P=0.019). In addition, NMD reduced more in the Leu7Pro7 genotype (-1.8±1.7 vs. -0.1±2.6 %, P=0.037). Changes in FMD were similar between the genotypes. However, positive correlation of IL-6 with 2-year change in FMD was found in Leu7Pro7 genotype (P<0.001) and negative correlation in Leu7Leu7 genotype (p=0.040). Patients with T2D and the Leu7Pro7 genotype have more rapid increase in brachial IMT and a decrease in brachial NMD compared to T2D patients with the Leu7Leu7 genotype, suggestive of increased rate of atherosclerosis.

T1:PO.15

Hypoxia Induces GLUT1, GLUT3 and GLUT5 Facilitative Glucose Transporter Expression (but not GLUT4, GLUT10 and GLUT12) in Human Adipocytes

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We have recently suggested that hypoxia may occur in enlarged adipocytes distant from the vasculature as adipose tissue mass expands in obesity, and that this leads to changes in the production of inflammation-related adipokines. Hypoxia can induce a range of metabolic adaptations in cells, including the augmentation of glucose utilisation. In initial studies, expression of the GLUT1 facilitative glucose transporter in adipocytes was rapidly increased by hypoxia; we have now examined the effects of low O 2 tension and chemically - induced hypoxia on the expression of other members of the GLUT transporter family expressed by human adipocytes.
Human adipocytes (15 days post -differentiation in culture) were exposed to 1% O 2 or 100 •M CoCl2 for up to 24 h, while control cells were maintained in 21% O 2 alone. mRNA levels of the GLUT1, GLUT3, GLUT4, GLUT5, GLUT10 and GLUT12 transporters were quantified by real -time PCR.
There was no significant change in the mRNA level of GLUT4, GLUT10 and GLUT12 in response to either 1% O 2 or CoCl2. However, there were substantial increases in GLUT1 (maximum 14-fold), GLUT3 (maximum 10 - fold) and GLUT5 (maximum 9 - fold) mRNAs in response to both low O 2 tension and chemically-induced hypoxia. These increases were particularly rapid for GLUT3, and were accompanied by an elevation in the cellular level of the key hypoxia-sensitive transcription factor, HIF-1•.
It is concluded that hypoxia induces rapid increases in the expression of facilitative glucose transporters in human adipocytes, consistent with increased glucose utilisation. The response is, however, selective to specific GLUTs.

T1:PO.16

Neuromedin beta: The polymorphism P73T in Czech obese and nonobese women

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Background and Aim: Recently, the association of the homozygous mutation T73T of neuromedin beta with increased body weight, BMI, waist girth and body fat, was published. Our pilot study determined the genotypic distribution of the *NMB* gene in obese/nonobese women and assessed possible associations of the minor allele T carriership with screened parametres.
Materials and Methods: Anthropometric and biochemical characteristics of 144/235 obese/nonobese women (age 51.0±11.0/31.84±11.53 years; BMI 37.13±7.17/23.24±3.89kg/m^2) were collected. For screening of P73T (c.217C>A) variant in the exon 2 of the *NMB* gene the single strand conformation polymorphism (SSCP) method was used.
Results: The genotypic distribution (PP/PT/TT) in obese women is 53.5/39.6/6.9 [%], in nonobese women 52.8/38.7/8.5 [%]. Frequency of TT genotype variant was higher in severely obese women with BMI•35 (8.2%) than in obese women with BMI<35 (5.2%). However, the differences were not significant. In nonobese women the carriers of homozygous genotype TT had significant higher level of glycosylated proteins (P=0.010, ANCOVA, adjustment for age).
Conclusion: No significant associations of P73T genotype with screened anthropometric parameters were observed in obese women. In nonobese women TT genotype was associated with higher level of glycosylated proteins. A tendency for association of severely obese with TT genotype should be evaluated in a larger cohort.
Supported by the grants IGA MHCR NR/7800-4 and NR/7809-5.

T1:PO.17

Regulation of adiponutrin expression by feeding conditions in rats is altered in the obese state

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Adiponutrin is a non-secreted adipose specific protein with triglyceride lipase and transacylase activities, regulated by changes in energy balance (increased expression after re-feeding) in rodents and up-regulated during adipocyte differentiation, that has been proposed to be involved in the maintaining of energy homeostasis.
The objective of this work is to characterise the effect of feeding conditions on adiponutrin expression behaviour in different rat adipose tissue depots under normal and obese conditions. Two rat models were used, Wistar (lean and overweight) and Zucker (lean and obese), submitted to fasting/re-feeding. Adiponutrin was determined in different white adipose tissue depots (epididymal, inguinal, mesenteric and retroperitoneal) and in interescapular brown adipose tissue by RT-PCR.
We have found site-specific differences in adiponutrin expression in different adipose depots. The depot-specific adiponutrin expression is similar in lean and obese animals, except in the inguinal depot, where adiponutrin is over-expressed in obese Zucker. Independently of the degree of expression in the tissue, adiponutrin is an acute sensor of feeding conditions: in lean rats, 14-h fasting greatly decreases adiponutrin mRNA levels in all the depots studied, while 3-h re-feeding allows the recovery of the levels found in control animals. In both overweight Wistar and obese Zucker rats, the decreased mRNA expression observed after fasting in lean rats is not as evident and, moreover, in the obese Zucker there is no recovery after re-feeding. We can conclude that adiponutrin expression is highly regulated by feeding conditions in the different adipose tissue depots, but this regulation is impaired in obese rats.

T1:PO.18

Oleoyl-estrone inhibits the expression of 17-beta-hydroxysteroid dehydrogenase in rat tissues

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Adult male overweight rats were treated for 10 days with oral gavages of 10 nmol/g oleoyl-estrone (OE), and compared with controls and pair-fed (PF). Day 10 lumbar WAT, testicles and adrenal gland RNA was used for real-time PCR amplification using primers for 17-beta-hydroxysteroid dehydrogenase isoenzymes 1,2,3,4,7,8 and 11. Testosterone, androstenedione, estradiol and estrone in serum were measured by HPLC-MS/MS. Testosterone and androstenedione levels decreased versus controls in OE (x1/10) and PF (x1/2); estradiol (x2) and estrone increased in OE (x4) and decreased in PF (x1/2). Enzyme expression (compared with controls) in testicle of OE was 70-95%, and PF 80-100%; in adrenals OE was 95–120% and PF 100–144%; in WAT OE was 35–74% and PF 32–82%. The androgen drop seems to be a consequence of lower expression (activity) of the enzyme in testicle and, especially, in WAT, affecting similarly odd and pair isoenzymes (i.e. both directions of the reaction). This limits the conversion to estradiol of excess available estrone (from OE hydrolysis), thus limiting the estrogenic effects. However, this also results in decreased testosterone synthesis. These effects are very close to those observed in PF, receiving no estrone load, which suggest that energy availability may play also a significant role in androgen/estrogen metabolism.
Disclosure M.M. Romero and R. Vilà are employees of the University of Barcelona spinoff Oleoyl-Estrone Developments SL, Barcelona, Spain; M. Esteve, J.A. Fernández-López and M. Alemany are shareholders of the same company
Financing sources
This study was supported by grants PI052179 of the "Fondo de Investigaciones Sanitarias", and BFU2005-00524 of the "Plan Nacional de Investigación en Biología Fundamental", both from the Government of Spain, as well by a research contract of Oleoyl-Estrone Developments SL, (Barcelona, Spain).

T1:PO.19

Beckwith-Wiedemann Syndrome: importance of neonatal follow up for early diagnosis

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Beckwith-Wiedemann Sindrome (BWS) is a congenital overgrowth syndrome: the incidence is about 1:13700 births, with an equal sex distribution. It's a clinically and genetically heterogeneous disorder. The phenotype of BWS is likely to result from an imbalance of a number of critical genes at chromosome 11p15. In BWS, 85% of cases are sporadic and 15% are autosomal dominant. In BWS, the most common phenotype are: macroglossia, abdominal wall defects, increased growth (birth weight and length, visceromegaly, hemihyperplasia, ear lobe crease). Our case report is about a 1 month old newborn female; any sort of problem was reported in family history and gestational anamnesis was completely negative. She was born at 38 week of gestational age with normal auxological evaluation except for weight (> 90° centile). Biochemical evaluation do not show any sort of pathological parameter. During follow up at 15 day and 1 month, we discovered an alteration of legs measure: in particolar left leg was lager than right one; for this reason, we begin a genetic evalutation with final diagnosis for BWS.This case report underlines the importance of newborn precocious clinical evalutation after birth durino first month of life: a normal newborn, sometimes, could mask an important clinical problem.

T1:PO.20

Tungstate antiobesity effects require intact leptin system

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Aims: Assess the role of leptin in the tungstate antiobesity effects.
Methods: *Ob/ob* and *lean* mice were treated with tungstate (180mg/Kg/day) for 30 days. Body weight (*BW*) and food intake (*FI*) were measured daily. Oxygen consumption was determined by indirect calorimetry. Epididymal adipose tissue (*epWAT*) from *lean* mice was transplanted subcutaneously into *ob/ob* mice and tungstate was administered for 4 weeks. An additional control group of *ob/ob* mice transplanted with *ob/ob* epWAT was used as a leptin deficiency control. Fat transplants were removed and tungstate treatment was carried out for additional two weeks.
Results: Tungstate treatment significantly reduced BW gain, adiposity and FI, and increased oxygen consumption in *lean* mice. However, in treated *ob/ob* mice none of these parameters were significantly changed. From second week onwards, *ob/ob* mice transplanted with epWAT from *lean* mice showed a decrease in BW gain compared to transplanted control mice, related to the onset and rise of plasmatic leptin levels. In addition, FI and oxygen consumption were reduced and increased respectively in these animals. These effects were amplified in transplanted *ob/ob* mice treated with tungstate. Moreover, morphological differences in brown adipose tissue were observed in transplanted untreated *ob/ob* mice, which are more accentuated in treated animals. Finally, the removal of transplanted adipose tissue from the treated mice immediately halted tungstate antiobesity effects, thus increasing BW and FI.
Conclusions: Tungstate require leptin to exert its antiobesity effects through increasing energy expenditure and reducing FI.

T1:PO.21

Polymorphisms in the APOA5-A4-C3 locus regulate plasma triglyceride levels and modulate the risk of metabolic syndrome in French populations

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We assessed whether the *APOA5* S19W, T-12,238C, *APOA4* T347S, and *APOC3* C-482T and C3238G (*Sst*I) polymorphisms may be associated with susceptibility to the metabolic syndrome and/or with the clinical variables related to the metabolic syndrome (waist circumference, blood pressure, plasma glucose, triglyceride and HDL-cholesterol levels). We genotyped for these 5 polymorphisms 3 population-based samples from the east (n=953), south (n=1082) and north (n=1103) of France including overall 932 individuals with and 2206 individuals without metabolic syndrome. We detected significant associations between the *APOA5* 19W (P<0.0001) and *APOC3* 3238G (P=0.02) alleles and higher plasma triglyceride levels. Moreover, the *APOA5* 19W allele conferred an increased risk of metabolic syndrome compared with S19S genotype (OR=1.30 [1.03–1.66]). Haplotype analysis revealed that the effect of the *APOA5* S19W polymorphism and *APOC3* C3238G polymorphisms on plasma triglyceride levels were independent from each other. Finally, there were statistically significant interactions (i) between waist girth, plasma triglyceride levels and the *APOA5* S19W polymorphism i.e. plasma triglyceride level differences between carriers and non-carriers of the 19W allele were more pronounced in subjects with abdominal obesity than in those with low waist girth, and (ii) between plasma insulin levels, plasma triglyceride levels and the *APOC3* C-482T polymorphism i.e. plasma triglyceride level differences between carriers and non-carriers of the -482T allele were more pronounced in subjects with insulin resistance than in those with low insulin levels. These data confirm that genetic variability in the APOA5-A4-C3 locus regulate -in a complex manner- plasma triglyceride levels and modulate the risk of metabolic syndrome in humans.

1. Conflict of Interest: None Disclosed.
2. Funding. Research relating to this abstract was funded by the Conseil Régional du Nord- Pas de Calais, ONIVINS, Parke-Davies Laboratory, the MGEN, Groupe Fournier, the Réseau National de Santé Publique, the Direction Générale de la Santé, the INSERM, the Institut Pasteur de Lille, the Unité d'Evaluation du CHU de Lille, the Centre d'Examen de Santé de Strasbourg, the CPAM de Sélestat and the Fédération Régionale de Cardiologie d'Alsace.

T1:PO.22

Metabolic and hepatic effects of chronic ethanol consumption in obese ob/ob mice

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Background: Recently, we shown that obese ob/ob mice receiving ethanol (2.5 g/kg) daily for 4 days exhibited TNF-alpha-dependent apoptosis in liver and some resistance to ethanolinduced oxidative stress when compared to lean mice (Robin *et al*., Hepatology 2005).
Aim of the study: To determine the effects of chronic ethanol intoxication (CEI) in obese mice.
Methods: C57BL/6J-ob-ob mice and their lean littermates received increasing amounts of ethanol in water reaching 21g/kg/d after 6 months.
Results: CEI did not induce hepatic cytolysis and decreased hepatic triglyceride levels by 20%. Moreover, CEI induced an important loss of body weight, and an apparent improvement of insulin resistance with a significant reduction of blood glucose and insulin. Although intoxicated ob/ob mice ate less than naïve ob/ob mice throughout the treatment, the loss of calorie intake was fully compensated with the calories supplied by ethanol. However, despite the lack of hepatic cytolysis, CEI caused a moderate oxidative stress in ob/ob liver with decreased aconitase and glutathione S-transferase activities and increased oxidized glutathione. CEI in lean mice did not affect body weight, liver triglycerides and blood glucose. Though hepatic oxidative stress was detectable in lean mice it was somehow reduced when compared to ob/ob liver.
Conclusions: CEI improves some metabolic disorders in ob/ob mice (e.g. fatness, insulin resistance, steatosis). Although CEI induces a moderate oxidative stress in liver, this was not accompanied by cytolysis. We are currently performing microarray analyses in liver to assess the expression of genes involved in fat and glucose homeostasis and oxidative stress.

T1:PO.23

Increased fatty acid oxidation in liver is involved in the body fat-lowering effect of trans-10,cis-12 conjugated linoleic acid in adult hamsters

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In previous studies we have demonstrated that one of the mechanisms underlying the reduction in body fat accumulation induced by *trans*-10,*cis*-12 conjugated linoleic acid (CLA) in young hamsters is an increase in both mitochondrial and peroxisomal fatty acid oxidation in liver. The purpose of the present work was to determine whether this CLA effect is maintained in adult hamsters, in which the body fat-lowering effect of CLA is lower than that observed in young hamsters. Sixteen male Syrian Golden hamsters (8-month-old) were divided into two groups and fed high-fat diets containing 0.5% linoleic acid (control group) or 0.5% *trans*-10,*cis*-12 CLA for 6 weeks. Liver triacylglycerol content, as well as carnitine-palmitoyl transferase I (CPT-I) and acyl CoA oxidase (ACO) activities were assessed by spectrophotometry. Hamsters fed the *trans*-10,*cis*-12 CLA-enriched diet showed an increase in liver size when compared with the controls (8.33±0.42 g *vs* 10.07±0.36 g; P < 0.01), not due to increased triacylglycerol accumulation (3.95±0.98 mg/g *vs* 3.65±0.30 mg/g). *Trans*-10,*cis*-12 CLA increased ACO activity (1.96±0.34 nmol/min/mg protein *vs* 3.12±0.48 nmol/min/mg protein; P < 0.05), but CPT-I activity remained unchanged (7.19±0.95 nmol/min/mg protein *vs* 8.12±0.14 nmol/min/mg protein). These results show that, in adult hamsters, liver is a target organ for *trans*-10,*cis*-12 CLA because this isomer increases hepatic microsomal fatty acid oxidation. Nevertheless, it is important to emphasize that the age has an influence on this effect because young hamsters showed a greater increase in peroxisomal fatty acid oxidation and also an increase in mitochondrial fatty acid oxidation after CLA feeding. Research relating to this abstract was funded by Ministerio de Educación y Ciencia (AGL2005-02494).

T1:PO.24

Increased cortisol level in type 1 diabetic patient may lead decreasing of bone mineral density

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Objective: In this study we aim to investigate the association of osteoporosis and type 1 diabetes in 43 type 1 diabetic subjects and 41 control subjectes.
Subjects and Methods: Bone mineral density of both groups were measured by DEXA. Age, BMI, waist/ hip ratio, daily calcium consumption were determined in both groups. Twenty-four hours urinary calcium, phosphorus, deoxipyridinoline and pyridinoline were measured. Osteocalcin ALP, IGF- 1, IBF-BP3. HbA1c, cortisol, albumin, LDL and triglyceride were measured in both groups. Independent t-test and chi- square test were used to compare the groups.
Results: Age, body weight, BMI, waist/ hip ratio, daily calci•um consumption of diabetics were not different from the control group (P>0.05). Total lumbal BMD (0.88±0.1; 0.93±0.1 g/cm² respectively; P<0.05) total femur BMD (0.93±0.14, 0,99±0.1 g/cm² respectively; P<0.05) and total femur Z- score (-0.16±1, 0.53±0.7 respectively; P<0.005) of the diabetic group were statically lower than control group. Urine DPD/ creatinine level (7.6±6.1, 4.9±3.8 pmol/µmol, respectively; P<0.05), serum ALP level (113±62, 74±18 U/L respectively; P<0.001), IGF-BP3 level (5.4 ±0.9, 4.7±1 µg/ml respectively; P<0.001) of diabetic groups were statically higher than control group. Serum cortisol levels in diabetic group were statically higher than control group (14.7±3, 12.8±2.7 µgr/dl respectively; P<0.005).
Conclusions: 1- Bone mineral density of type 1 diabetic patient were decreased due to increased bone turnover
2- Increased cortisol level in type 1 diabetic patient may lead decreasing of bone mineral density.

T1:PO.25

Regulation of caveolin-1 association with adipocyte lipid droplets

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Caveolins, proteins coating cell surface caveolae, have been also located on intracellular lipid droplets (LD) but the significance of this association remains unknown. In 3T3-L1, rat or human adipocytes, we have found that LD-associated caveolin-1 was organized as oligomers with similar electrophoretic mobilities but different detergent resistance properties than caveolin-1 found in caveolae. During adipocyte development, a process can be followed in vitro using 3T3-L1 adipocytes, we observed that the coating of LD with caveolins was delayed relative to that of perilipin, a major LD marker, and dependent on Src kinase activation. The regulation of LD-associated caveolin content was also studied in physiopathological conditions where adipocytes exhibited large variations in their lipid stores. We used enlarged adipocytes isolated from obese Zucker rats, and human isolated fat cells from patients exhibiting wide range variations in their adiposity. Despite no change in total caveolin-1 expression, a dramatic increase in LD caveolin-1 concentration was found in obese *vs* lean Zucker rat adipocytes. Moreover, caveolin-1 content of LD positively correlate with fat cell size in human adipose tissue samples, pointing out fat cell size as an important regulator of the association of caveolin-1 to LD. All together, this study revealed an original organisation of caveolin on LD, differing from what found in caveolae. Furthermore, changes in caveolin content of LD in chronic situations of altered adipose tissue mass suggest that this protein might be involved the management of lipid stores.

T1:PO.26

Effects of different fatty acids and dietary lipids on adiponectin gene expression in 3T3-L1 cells and C57BL6 mice adipose tissue

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The type of lipid may play a causal role on the onset of obesity-related pathologies. The main protein secreted by the adipose tissue is adiponectin, which has anti-atherogenic and anti-diabetic properties. The aim of this study was to evaluate the effects of four different high fat diets (enriched 15% with soybean oil -S, fish oil-F, coconut oil -CC or lard-L) on the adiponectin adipose tissue mRNA and secretion by mice fed for either 2 days (acute treatment) or 60 days (chronic). Also, 3T3-L1 cells were treated for 48 hours with 250 μM of one out of six different fatty acids: palmitic, linoleic, eicosapentaenoic (EPA), docosahexaenoic (DHA), lauric or oleic acid. Adiponectin serum concentrations were decreased in the S, CC and L chronically and acutely treated animals. Adiponectin mRNA decreased in the retroperitoneal adipose tissue after acute treatment with all fatty acids rich diets, in epididymal adipose tissue of chronic S and CC and in 3T3-L1 cells treated with palmitic, linoleic, EPA and DHA. Conclusion: according to the type of fatty acid in the diet, it can occur reduction of adiponectin serum levels and adipose tissue gene expression, which also seems to be a time and tissue-specific response. The enrichment of F produced a transitory reduction on adiponectin serum concentration. However, the enrichment with L, S or CC, in different manners reduced the adiponectin serum concentration. It is possible that these diets, may lead in the long run to the development of insulin resistance and atherosclerosis. Research relating to this abstract was funded by FAPESP and CAPES.

T1:PO.27

Expression of inflammation-related adipokines by canine adipocytes differentiated in primary cell culture: response to TNF-α

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Obesity is now the most common nutritional disorder of companion animals . A role for inflammation-related adipokines in the development of obesity -associated diseases in humans is increasingly recognised. TNF-•, a pleiotropic proinflammatory cytokine, has been shown to play an extensive role in ad ipose tissue function. The quantitative expression profile (qPCR) of a series of in flammation-related adipokine genes was examined during the differentiation of canine preadipocytes to adipocytes in culture. The effects of TNF-• on the expression of these adipokines wa s also investigated. No significant difference was found in inflammatory adipokine mRNA levels between five major canine white adipose tissue depots (subcutaneous, perirenal, omental, gonadal and falciform ligament). Leptin and adiponectin expression was highly differentiation -dependent. NGF, a target derived neurotropin, was found to be expressed in c anine WAT. Its expression pattern was found to mirror that of other inflammation -related adipokines such as IL-6 and TNF-•, with levels being higher early in the di fferentiation process. Treatment of differentiated canine adipocytes with TNF -• (5 or 50 ng/ml for 2 or 24 h) significantly reduced the mRNA level of leptin (acute –2 h) and adiponectin (chronic –24h). In contrast, TNF -• induced substantial increases in several adipokines mRNAs including IL-6 (18-fold), MCP -1 (17-fold) and NGF (4 -fold), the largest increase being in TNF-• itself (1868 -fold).
These results demonstrate that there are significant qua ntitative changes in the level of adipokine gene expression during differentiation in canine adipocytes and that TNF -•exerts a pleiotropic effect on adipokine production in the dog. Payment received from the BBSRC
Research relating to the abstract was funding by both the BBSRC and WALTHAM Centre for Pet Nutrition.

T1:PO.28

Inhibition of 3T3-L1 adipocyte differentiation by oxidized-ldl: enhanced ccaat/enhancer-binding protein α mRNA and protein levels

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Adipocyte differentiation occurs through temporal pattern of expression of adipogenic genes that give rise to the adipocyte phenotype. Abnormal regulation of adipocyte differentiation is linked to pathological conditions such as obesity and diabetes. Oxidised low-density lipoproteins (ox -LDL) play a critical ro le in the regulation of cell proliferation, apoptosis and differentiation and are associated to obesity.
Aim of the study was to investigate the effects of ox-LDL on the differentiation of 3T3 -L1 preadipocyte s cell line. To this end, in the presence or absence of ox –LDL (0.05mg/ml), postconfluent 3T3 -L1 preadipocyte s were induced to differentiate by standard ho rmonal mixture. In both the experimental conditions, during the progression of differentiation, we evaluated:1) cell proliferation by 3H -thimidine incorporation, 2) induction of apoptosis by Annexin V analysis, and 3) expression of C/EBP • both at RNA and protein level by RT -PCR and western blotting respectively. Our results showed that ox-LDL, significantly, 1) inhibited differentiation without affecting cell proliferation 2) did not induce apoptosis in 3T3 -L1 cell line, as observed in se veral different cell types, 3) and caused a sustained up -regulation, up to five days after differentiation induction, of C/EBP • mRNA transcription associated to an increased nuclear accumulation of C/EBP • protein. It is known that o x-LDL have mitogenic e ffects and that C/EBP • is a promitotic factor, thus our data suggest that ox-LDL are involved in the dysregulation of the adipose tissue homeostasis by affecting the number of preadipocytes due, at least partially, to altered levels of C/EBP •.

T1:PO.29

Sub-strata of subcutaneous abdominal adipose tissue (Sat): key role in the insulin sensitizing effects of rosiglitazone

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Objective: Abdomial visceral (VAT) and sub-cutaneous adipose tissue (SAT), with SAT comprised of superficial-SAT (sSAT) and deep-SAT (dSAT), are metabolically distinct. Anti-diabetic agents thiazolidinediones (TZDs), in addition their insulin-sensitizing effects, re-distribute SAT suggesting TZD- action involves adipose tissue strata-specific regulation. We investigated the expression of proteins key to adipocyte metabolism in rosiglitazone treated preadipocytes, to establish a role for the diverse strata of abdominal adipose tissue in the insulin-sensitizing effects of TZDs.
Methods: Adipocytes and preadipocytes were isolated from sSAT, dSAT and VAT samples obtained from 5 normal to over-weight subjects (BMI 24.5±2.04kg/m^2; age 58.4±5.7yrs). Preadipocytes untreated (U) or treated with a differentiation cocktail (DI) including rosiglitazone (DIR) for 9days, were evaluated for strata-specific differences in differentiation, including peroxisome proliferatoractivated receptor (PPAR) and lipoprotein lipase (LPL) expression, insulin sensitivity via adiponectin, glucose transport-4 (GLUT4) and resistin, glucocorticoid metabolism with 11 - hydroxysteroid dehydrogenase type-1 (11 HSD1) and alterations in the adipokine leptin.
Results: While strata-specific differences were absent with the classic differentiation cocktail, with rosiglitazone sSAT had the most potent response followed by dSAT, while VAT was resistant to differentiation. With rosiglitazone, universal strata effects were observed for PPARγ, LPL, resistin and leptin, with VAT in all cases except resistin, expressing significantly lower expression levels. Clear dSAT-specific changes were observed with decreased intracellular GLUT4. sSAT-specific alterations included decreased 11 HSD1, while secreted adiponectin was potently upregulated with respect to dSAT and VAT.
Conclusions: The sub-strata of SAT, sSAT and dSAT, appear to be key to the metabolic changes that arise with rosiglitazone administration.

T1:PO.30

Differential lipolytic regulation in human embryonic stem cell-derived adipocytes

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Objective: Human embryonic stem cells (hESCs) have raised great hopes for future clinical applications. Several groups have succeeded in differentiating hESCs into adipocytes as determined by morphology, mRNA expression and protein secretion. However, determination of lipolytic response, the most important characteristic of adipocytes, has not been performed. The present work intended to study adipogenic conversion of hESCs by functional assessment of differentiation.
Research Methods and Procedures: Single undifferentiated colonies were allowed to transform into embryonic bodies (EB). Messenger RNA expression for a set of adipocytespecific genes as well as leptin/adiponectin secretion and lipolysis were assessed at different time points following differentiation.
Results: In contrast to primary human adipocytes, hESC–derived adipocytes showed a very small response to classical -adrenergic agonists although they expressed the major genes in the lipolytic cascade. In contrast, there was a significant lipolytic response to atrial natriuretic peptide.
Discussion: Although ESC-derived adipocytes appear to be morphologically and expressionally similar to mature adipocytes there are important functional differences which could depend on their early developmental origin. We conclude that, in contrast to mature adipocytes, hESC-derived adipocytes display a differential response to ANP and catecholamines.

T1:PO.31

The relationship between several genetic polymorphisms and reduction therapy in Czech obese youth population

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The relationship among polymorphism alleles of selected genes with the degree of obesity, as well as of the effect of reduction therapy in a spa (5–6 weeks, diet and exercise) in Czech obese subjects (42 obese boys and 72 girls) was analyzed, using Mann-Whitney test. In the carriers of PPARgamma 2, polymorphism Pro12Ala no statistically significant associations with biochemical and anthropometric parameters were found. In obese girls no statistically significant relationships with biochemical and anthropometric parameters and their changes after reduction therapy were observed as related to UCP1, polymorphism A-3826G gene. In obese boys, in carriers of rare genotype GG a lower body weight decrease and a smaller increase of the percent of muscle tissue were found as compared to common genotype AA. As related to polymorphism Gln27Glu in gene Beta2AR, no significant associations among anthropometric and biochemical parameters characterizing glucose and lipid metabolism were revealed. Male homozygotes Glu2/Glu had smaller percentual decrease of abdomen circumference as compared to homozygotes Glu/Glu. Female homozygotes Glu/Glu had significantly lower percentual decrease of body weight as compared to heterozygotes. The presence of Glu allele in genotype was associated with lesser effect of reduction therapy in both genders. The presence of long alleles in genotype INS VNTR could have a positive effect on weight reduction in girls, not in boys. Polymorphism Ala 54 Thr in Gene FABP2 had no association with biochemical parameters of insulin secretion and lipid metabolism. Genotype Thr/Thr can lower the effectiveness of reduction therapy in girls, not in boys. The study was supported by a research grant of the Ministry of Education, Youth and Sports of CR, grants No. MSM 0021620843 and 1M06014.

T1:PO.32

Effects of resveratrol on human adipocyte biology

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Objectives: Calorie restriction (CR) leads to retardation of the aging processes and to longer life in many organisms. The effect of CR can be mimicked by certain natural plant products, e.g. some flavonoids and most efficiently by resveratrol, a sirtuin activating compound, which is present in grapes and red wine. This intriguing finding raises the question whether simply taking a chemical would counteract all the bad effects of being overweight without changing eating habits. In this study, we have investigated the effects of resveratrol on human fat cell biology.
Methods: As a model system we have used human SGBS preadipocytes and adipocytes. Adipogenic differentiation was determined by morphological means. Apoptosis was studied by flow cytometry. In addition, de novo lipogenesis and effects on gene expression were investigated.
Results: Resveratrol inhibits human preadipocyte proliferation and, under special conditions, induces apoptosis in preadipocytes and adipocytes. Adipogenic differentiation was inhibited in a time- and dose-dependent manner. Incubation with low doses of resveratrol resulted in suppression of de novo lipogenesis. In addition, incubation with resveratrol differentially influenced gene expression of adipocytokines. While expression of leptin and adiponectin was unaffected, IL-6 and IL-8 were significantly down-regulated.
Conclusions: Taken together, our data show that resveratrol inhibits human preadipocyte proliferation, conversion into mature adipocytes, and de novo lipogenesis, and induces apoptosis under special conditions. In addition, it inhibits production of cytokines which are involved in the development of obesity-related disorders. These data suggest that resveratrol might exert positive effects on adipocyte biology *in vivo.*
2. Funding: Research relating to this abstract was in part funded by the Deutsche Forschungsgemeinschaft (DFG WA 1096/3-2).

T1:PO.33

Conjugated linoleic acids promote human fat cell apoptosis

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Conjugated linoleic acids (CLA) are conjugated dienoic isomers of linoleic acid. Some isomers have been shown to reduce fat mass in animal and cell culture models. However, controversial results were obtained in studies of CLA supplementation in human subjects. In order to get more insights into the direct effects of CLA on human fat cells, we have studied the influence of c*is*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA on the biology of human SGBS preadipocytes and adipocytes. Both CLA isomers equally inhibited the proliferation of preadipocytes in a dosedependent manner. Continuous treatment with 1-10 μM *trans*-10, *cis*-12 CLA, and to a weaker extent c*is*-9, *trans*-11 CLA inhibited accumulation of lipids during adipogenic differentiation. Treatment with higher doses of CLA induced apoptosis in preadipocytes, in differentiating cells, and adipocytes. The *trans*-10, *cis*-12 isomer had a higher apoptotic potency in adipocytes than *cis*-9, *trans*-11 CLA. Taken together, the treatment of human preadipocytes and adipocytes with physiological relevant concentrations of CLA resulted in an impairment of proliferation and differentiation and induction of apoptosis. The *trans*-10, *cis*-12 isomer was more potent than the *cis*-9, *trans*-11 isomer. Further clinical studies are needed to evaluate the effects of CLA on human fat mass and metabolism *in vivo*.

2. Funding:
Research relating to this abstract was in part funded by the Deutsche Forschungsgemeinschaft (DFG WA 1096/3-2).

T1:PO.34

Apelin gene expression of white adipose tissue is regulated by cafeteria diet and vitamin C supplementation in rats

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Objective: To study the apelin mRNA expression in white adipose tissue (WAT) from high-fat diet obese rats with or without vitamin C (VC) supplementation, in order to analyze the role of this antioxidant vitamin in obesity markers and related risk factors.
Methods: Three groups of male Wistar rats (n=25) were fed on a standard pelleted (Control group) or a high-fat diet during 56 days in the absence (Cafeteria group) or presence of oral supplementation (750 mg/kg of body weight) of VC (VC group). At the end of the experimental period, body composition and WAT gene expression of apelin, leptin, IRS3, and IL1ra were analyzed.
Results: Animals fed on a high fat diet increased their body weight, total body fat and the different WAT tissues weights as compared to controls (P<0.01). Interestingly, VC supplementation reduced weight gain (P<0.05) and WAT depots weight (P<0.05). Subcutaneous (Sc) apelin mRNA expression was higher in Cafeteria than Control groups (P<0.01) and this increase was reversed by dietary VC supplementation (P<0.05). Moreover, significant associations between Sc apelin gene expression and almost all the studied variables were found, being of special interest the correlations with serum leptin (P<0.05, r=0.517), HOMA (P<0.05, r=441), liver MDA (P<0.05, r=0.478), and retroperitoneal leptin, IRS-3 and IL-1ra mRNA expression (P<0.001, r=0.701; P<0.001, r=0.692; and P<0.01, r=0.563).
Discussion: It has been shown that vitamin C could be a protective factor of cafeteria induced overweight. Clear associations between apelin mRNA expression from Sc WAT and several markers of adiposity, insulin resistance, liver oxidative stress and inflammation were observed, suggesting an important role for apelin in the excessive weight gain induced by high-fat diet in rats.

T1:PO.35

AGTR1 gene expression is down-regulated in obese compared to lean young male high-fat consumers

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Objective: The aim of the present study was to compare the AGTR1 gene expression in subcutaneous abdominal fat from lean vs. obese high fat intakers with a similar physical activity patterns.
Subjects and methods: Eighteen young men, 9 lean (BMI=23.1±0.4 kg/m^2 and 9 obese (34.7±1.2 kg/m^2) with a similar habitual dietary intake of fat (44.6±2.2 vs. 42.5±1.8% daily energy from fat and physical activity (17.5±5.1 vs. 18.0±4.4 METsh/week) for lean and obese, respectively), were recruited. Subcutaneous abdominal fat biopsies were obtained and total RNA was extracted, purified and probed into Affymetrix GeneChip Human U133A. The microarray data was verified by Real-time PCR (ABIPRISM 7000).
Results: Both the microarray and real-time PCR analysis revealed that AGTR1 gene was down-regulated in subcutaneous adipose tissue of obese compared to lean subjects. Moreover, we observed a significant association between AGTR1 gene expression and the QUICKI index: (P<0.05, r=0.58).
Discussion: The decreased expression of AGTR1 gene in subcutaneous adipose tissue of obese subjects and their correlation with the lower insulin sensitivity suggest that renin-angiotensin-system (RAS) could be involved in the susceptibility to develop obesity and insulin resistance when eating a high fat diet.

T1:PO.36

A common polymorphism (–3185C>T) in the promoter of the visfatin gene (PBEF1) influences plasma insulin levels in a Greek women population

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Visfatin (*PBEF1*) is a novel insulin-mimetic adipocytokine which has been suggested to play a role in glucose homeostasis and may represent a link between central obesity and insulin resistance. In the present study we explored potential associations between two common single nucleotide polymorphisms in the promoter of the visfatin gene (*rs*11977021: –3185C>T and *rs*1319501:–423A>G) and adiposity-related phenotypes in 345 non-diabetic women (mean age: 47.0 ± 12.1 y) with a wide spread of BMI ranging from 18.5 to 47.4 kg/m^2 (mean: 29.0 ± 5.5 kg/m^2). We did not find statistically significant differences in genotype distributions or allele frequencies for the two polymorphisms between normal weight, overweight and obese women (minor allele frequencies in whole group: –3185T=0.27; –423G=0.24). Moreover, no significant associations were observed between any of the two polymorphisms with body composition (assessed by anthropometry and dual energy x-ray absorptiometry), fasting plasma lipid or glucose levels, except for a weak association between the –3185C>T polymorphism and plasma insulin levels (P=0.05). In multivariate analysis controlling for potential confounders, carriers of the minor –3185T allele had lower fasting insulin levels compared with carriers of the –3185C/C genotype (8.04 ± 3.88 mIU/L *vs.* 9.31 ± 5.30 mIU/L, P=0.04) and had lower homeostasis model assessment of insulin resistance (HOMA-IR) index (1.90 ± 0.96 *vs.* 2.23 ± 1.35, P=0.05). These data suggest that variation in the promoter region of the visfatin gene may not play a major role in the development of obesity in humans; however, the –3185C>T polymorphism may be associated with insulin resistance.

T1:PO.37

Association of the +45T>G and +276G>T polymorphisms in the adiponectin gene with insulin resistance in non-diabetic Greek women

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In this study we examined the associations between two single nucleotide polymorphisms (SNPs) in the adiponectin gene (+45T>G and +276G>T) and risk factors of type-2 diabetes in 350 non-diabetic Greek women (mean age: 47.0 ± 12.1 y, mean BMI: 29.5 ± 5.6 kg/m2). No association of the +45T>G or the +276G>T SNP with BMI, body composition variables and plasma lipid levels was observed. However, in multivariate analysis controlling for potential confounders both SNPs were found to be associated with insulin resistance (assessed by HOMA-IR index, $P<0.05$). In particular, carriers of the rare +45G allele exhibited lower fasting plasma insulin levels than did homozygotes for the +45T allele (7.88 ± 4.15 mIU/L *vs.* 8.89 ± 4.76 mIU/L, $P=0.009$) and had lower HOMA-IR (1.86 ± 0.99 *vs*. 2.12 ± 1.23, $P=0.01$). At position +276, carriers of the T allele had higher fasting insulin levels and higher HOMA-IR compared to +276G/G homozygotes (9.40 ± 5.32 mIU/L *vs.* 8.07 ± 3.96 mIU/L, $P=0.003$ and 2.25 ± 1.34 *vs.* 1.90 ± 1.00, $P=0.002$, respectively). SNPs 45 and 276 were not in linkage disequilibrium ($R = 0.26$) and haplotype analysis revealed that carriers of the +45T/+276T haplotype had significantly higher insulin levels and HOMA-IR than did non-carriers (9.40 ± 5.32 mIU/L *vs.* 7.99 ± 3.78 mIU/L, $P=0.002$ and 2.25 ± 1.34 *vs.* 1.88 ± 0.97, $P=0.002$, respectively). We conclude that the +45T>G and +276G>T polymorphisms in the adiponectin gene modulate insulin sensitivity and that haplotype +45T/+276T is a risk haplotype for insulin resistance in Greek women.

T1:PO.38

Ranking of redox potential between CHD risk factors. Plasma ischemia modified albumin (IMA), hsCRP and Chlamydia pneumoniae seropositivity in obese/metabolic syndrome patients

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About fifty percent of patients presenting acute coronary syndrome who does not display classical risk factors, remain inaccessible for prevention. Plasma hsCRP and ischemia modified albumin (IMA) were recently introduced as more informative cardiovascular risk markers. The intracellular bacterium *Chlamydia pneumoniae* is involved in the inflammation process of atherosclerosis.
Aim of study: was to assess the relation between *C. pneumonie* infection, plasma hsCRP and IMA level and oxidative stress/antioxidant plasma capacity.
Methods: A total of 83 men included in the study consisted of: cardiovascular event-free obese (BMI >30; < 35kg/m^2) men (O) without others CHD risk factors, and the control group (C) of healthy subjects. The lipoprotein profile, plasma hsCRP, IMA, markers of oxidative stress, antioxidant plasma capacity, and *C.pneumoniae* antibodies were measured.
Results: Plasma hsCRP and IMA/alb directly correlated with the body fat in whole group. Examination of high score IMA/alb ratio and hsCRP patients compared to reference group revealed low antioxidant level, oxidative alteration of LDL, higher plasma content of LOOH, TBARS, and reduced redox compensation index. Percentages of subjects from C and O groups positive for *C.pneumoniae* antibodies were associated with IMA/alb ratio in the highest group quartile. Markedly decreased plasma antioxidant capacity and increased oxidative stress characterized antibodypositive subjects.
Conclusion: Plasma hsCRP, *C.pneumoniae* antibody index as well as IMA score may serve as potential marker of oxidative stress and low antioxidant status.

T1:PO.39

Comparison of luminex and ELISA techniques for measuring circulating IL6, TNF? and MCP-1 in obese subjects

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Aims: Multiplex techniques provide the opportunity to screen a large number of proteins using a small sample volume, but results and precision relative to the standard ELISA technique is not clear. This study compares results for 3 inflammatory markers, obtained using the two techniques.
Methods: 74 subjects, recruited from the Cambridge area, with fasting blood samples were included in the study. IL6, TNF α and MCP-1 were measured using an ELISA technique (R&D Systems, Minneapolis, MN, USA) and a bead -based luminex technique (BioRad, Hercules, CA, USA). Serum C reactive protein (CRP) was measured as an independent index of inflammation (high sensitivity assay, Dade -Behring, Walton, UK). Bland Altman analysis was used to assess the agreement between the two methods. Correlation was used to further explore the relationship between the 3 markers of inflammation.
Results: Baseline TNFα was undetectable in 63 subjects using the luminex method. Scattergraphs and Bland Altman plots show poor agreement between the two methods (correlation coefficients for each of the 3 inflammatory markers (IL6 r=-0.11, TNFα r=0.16, MCP-1 r=0.42). None of the luminex results were significantly correlated with CRP concentrations, and while ELISA TNF α and MCP-1 were not correlated with CRP, IL6 was significantly related (IL6 r= -0.45, $P<0.01$).
Conclusions: While the multiplex approach has the advantage of providing a large number of results within a single test, the results obtained do not correlate well with standard ELISA assays, an d do not show the expected relationships with other measures of inflammation.
1. Conflict of Interest:
None Disclosed.
2. Funding
Research relating to this abstract was funded by the Medical Research Council.

T1:PO.40

The G1422A variant of the cannabinoid receptor gene (CNR1) is associated with abdominal adiposity in obese men

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Introduction: Recent data suggest that the endocannabinoid system is a key circuit controlling food intake and the cannabinoid receptor (CB1) has emerged as a target for pharmacotherapy for obesity. With this study, we wanted to assess whether *CNR1* G1422A genotype contributes to the development of obesity in the Belgian population. Furthermore, we investigated whether the G1422A variant is associated with obesity parameters in adult obese individuals.
Patients and methods: 161 healthy controls and 1093 obese subjects were included in this study. Anthropometric measurements were obtained and fat mass was assessed by bio-impedance. *CNR1* genotypes were analysed by TaqMan. Kruskall-Wallis and Mann-Whitney-U tests were performed for different endophenotypes, before and after controlling for age and BMI.
Results: The G1422A allele frequencies did not differ significantly between cases and controls (OR 0.94; $P=0.619$). Among premenopausal obese women, no significant associations between genotype groups and anthropometric parameters could be detected. In obese men, *CNR1* genotype was significantly associated with WHR, both unadjusted ($P=0.022$) and adjusted for age and BMI ($P=0.032$). Fat mass percentage also showed an association ($P=0.007$), which disappeared after adjusting for age and BMI.. Waist was associated with *CNR1* genotype ($P=0.017$) after the same adjustments. A trend for an association with fat mass was also observed (unadjusted p=0.088; adjusted $P=0.066$).
Conclusion: Our data do not indicate that the G1422A polymorphism in the cannabinoid receptor-1 gene is involved in the pathogenesis of obesity in our study population. The variant is, however, associated with increased abdominal adiposity in obese men.

T1:PO.41

Two novel mutations in the melanocortin-4 receptor associated with early-onset obesity

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Introduction: Obesity is a major health problem in industrialized countries. In a minority of cases obesity has been associated with a mutation in one gene. Currently, six such monogenic forms of obesity have been described. Melan ocortin-4 receptor deficiency is most common and the goal of the present study was to investigate whether pathogenic melanocortin-4 receptor (*MC4R*) mutations are a common cause of early -onset obesity in Belgium.
Patients and methods: A total of 165 obese children and adolescents were screened for mutations in *MC4R* with the WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Inc.). Direct sequencing was performed when the chromatogram deviated from the WT pattern.
Results: DHPLC screening identified 7 patients with a variation in *MC4R* (frequency = 4 .24%).Two adolescents harboured the common Val103Ile polymorphism and two other patients contained the Ile251Leu polymorphism. One obese child was heterozygous for the Thr112Met polymorphism. Additionally 2 novel amino acid changes in *MC4R* were discovered. One obese child carried a Phe280Leu substitution, while a second patient harboured a Pro260Gln change. Both these children suffer from obesity from an early age. These variants were not found when screening 100 healthy controls.
Conclusion: Screening of children and adolescents with early -onset obesity revealed two novel mutations in *MC4R*. Further functional characterization is necessary to fully understand the pathogenic effect of these mutations.

T1:PO.42

Sleep Duration and Childhood Obesity Prevalence

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The aim of this study was to determine whether an association between sleep duration and obesity exists in a sample of Portuguese children. A cross-sectional study of children 7 to 9.5 y old was performed between October 2002 and June 2003. A total of 2274 girls and 2237 boys were observed. Weight and height were measured, and parents filled out a questionnaire about family characteristics. Overweight and obesity, using age- and sex-specific body mass index (BMI) cut-off points as defined by the International Obesity Taskforce, were used. In the total sample we found 20.3% of overweight children and 11.3% of obese children. The prevalence of obesity (including overweight) decreased by duration of sleep: > 8 h, 49.1%, 8–9 h, 33.0%, 9–10 h 28.7 % and > 11 h, 26.3%. Parental education showed positive relationship with sleeping: children of more educate families showed more hours of sleeping; more TV hours showed a negative relationship with sleeping: more hours of TV less hours of sleeping; more physical activity showed a positive association with sleeping: more hours of physical activity more hours of sleeping. After adjusted for demographic factors such as parental obesity, parental education and TV, the odds ratio were: for sleeping 9–10 h, 1.07 (95% CI 1.06–1.07); 8–9 h, 1.14 (95% CI 1.11–1.16) and < 8 h, 2.28 (95% CI 2.27–2.29). The effect of sleep duration on childhood obesity is strong and seems to be independent of other risk factors associated with childhood obesity.

T1:PO.43

Towards leptin-derived peptides in cancer therapy: Partial agonists acting on the leptin – leptin receptor interface

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Obesity significantly increases the risk of cancer development, possibly through increased expression of leptin. High local leptin levels might promote carcinogen esis by inducing mitogenic cell response and thus, targeting leptin signaling represents a nov el cancer treatment option. Leptin mutants were shown to antagonize the protein's activity in vitro, but the lack of clinical success with full -sized leptin prompted us to search for a peptide - based leptin -receptor antagonist. Individual arms of the three bivalent receptor -binding leptin fragments, their reportedly antagonist analogs, as well as a synthetic construct comprising both surf aces of site II were synthesized and their ability to stimulate MCF -7 mammary carcinoma, DU -145 prostate carcinoma and M CF-10 normal mammary epithelial cells were studied. While none of the peptides influenced the proliferation of normal cells, the site II construct and an alanine mutant of site III reversed leptin -induced growth of cancer cells in the 10 – 100 nM concentration range. However, at a 10 -fold increased concentration, the same peptides showed growth promoting activity without exogenous leptin addition, suggesting that these leptin fragments are partial agonists rather than true antagonists. In addition, the more drug-like site III derivative underwent rapid exopeptidase cleavage, prompting us to design a second generation of site III analogs containing non -natural amino acid residues incorporated into strategic positions throughout the peptide. These leptin derivatives serve as lead compounds for further medicinal chemistry operations in order to develop a true leptin-receptor antagonist in cancer therapy of obese patients.

T1:PO.44

Water extracts of Paecilomyces tenuipes inhibit 3T3-L1 adipocyte differentiation induced by cathepsin

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Cathepsin S (CTSS) is a cysteine protease effecting on extracellular matrix (ECM) remodeling. Recently, several studies have reported that CTSS was an enzyme involved in obesity. CTSS is produced by mouse and human adipose cells. And CTSS gene expression is more elevated in adipose tissue from obese mouse than lean one. CTSS promotes preadipocyte differentiation by degrading fibronectin, a key component of preadipocyte ECM. Water extracts o f *Pacilomyces tenuipes* inhibited CTSS activity. In this study, inhibitory effect of *P. tenuipes* was examined with the CTSS specific substrate Z-Val-Val-Arg-AMC. Supplementation of *P. tenuipes* in 3T3-L1 cell media obviously reduced lipid droplet accumulation and adipogenesis induced by CTSS. Furthermore, *P. tenuipes* decreased the weight gain, subcutaneous adipose tissue growth, and serum glucose level in mice fed a high -fat diet. These studies suggest that *P. tenuipes* works on adipose cathepsin S, and presumably contributes to anti -obese activities.

T1:PO.45

High expression of osteopontin and CD44 is associated with severe hepatic steatosis in morbidly obese patients

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Osteopontin (OPN), a cytokine involved in various inflammatory diseases, plays an important role in the progression from liver steatosis to Nonalcoholic steatohepatitis (NASH) in mouse models of Nonalcoholic fatty liver diseases (NAFLD). We determined the hepatic gene expression levels of OPN and its cellular receptor CD44 in obese patients with or without steatosis and steatohepatitis. Liver biopsies were obtained from 47 severely obese patients undergoing bariatric surgery and 6 control subjects. Steatosis was graded 0 to 3 based on the percentage of hepatocytes with micro- or macro-vesicular steatosis (S0, none; S1< 30%; S2, 30–60%; and S3>60%). Among obese patients, 39 had steatosis alone (6 S0, 13 S1, 9 S2, 11 S3) and 18 had NASH (1 S1, 6 S2, 11 S3). OPN and CD44 gene expression was determined by real-time quantitative PCR. In patients with severe steatosis (S3), the OPN and CD44 gene expression was strongly increased compared with controls or obese patients without steatosis (S0). These high expression was similar in severe steatosis without or with steatohepatitis. The OPN protein expression, evaluated by immunoblotting, was also related to the severity of steatosis. Finally, OPN and CD44 gene expression was correlated with circulating alanine amino-transferase levels (an index of liver alteration) and insulin resistance. The progression from moderate to severe steatosis, which could be considered as a pre-NASH state, was accompanied by an upregulation of OPN and CD44 expression, which, in association with insulin resistance, could contribute to the pathogenesis of NAFLD in severely obese patients.

T1:PO.46

Plasma levels and adipose tissue expression of retinol-binding protein 4 are reduced during diet-induced weight loss in obese subjects but are not associated to changes in insulin sensitivity

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Background: Retinol-binding protein 4 (RBP-4) is produced by adipose tissue and has been suggested to play a role in the development of insulin resistance.
Aim: To investigate whether a diet-induced weight loss associated with improvement of insulin sensitivity induces changes in RBP-4 expression in adipose tissue and plasma levels. Methods: 24 obese women (body weight 97.3±15.7kg) were studied at basal conditions, after a 4 week very low calorie diet (VLCD) and 5-6 months later during the weight-maintenance phase (WM). Insulin resistance was assessed with euglycemic hyperinsulinemic clamp. Plasma and subcutaneous adipose tissue mRNA levels of RBP-4 were measured by ELISA and RT-qPCR, respectively.
Results: The weight loss was 7.4±1.7kg and 10.4±4.6kg after VLCD and at WM, respectively. Glucose disposal increased by 34.9% after VLCD and 57.5% at WM compared to the basal state. Plasma levels of RBP-4 were reduced after VLCD and at WM (B: 27.4±7.2; VLCD : 20.1±5.1; WM: 23.0±5.5µg/ml, P<0.01). Adipose tissue mRNA levels decreased 1.5 fold during VLCD and re-increased during WM. No correlations between diet-induced changes in RBP-4 production and insulin sensitivity indices were found.
Conclusion: The insulin sensitizing weight loss program promotes a reduction of RBP-4 plasma levels and adipose tissue expression. The study did not bring a direct evidence of the role of RBP-4 in the regulation of diet-induced changes in insulin sensitivity. The study was supported by grant GACR 04/0158 and EU program HEPADIP.

T1:PO.47

The Arg223Arg genotype of the leptin receptor gene is associated with obesity in Hungarian children

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Aim: The Gln223Arg polymorphism of the leptin receptor (LEPR) gene has been suggested to be associated with obesity and obesity related disorders. In this study we investigated the effects of the Gln223Arg polymorphism in LEPR gene on body composition variables in obese and normal weight children.
Methods: Genotyping was performed in 261 obese (O) and 131 normal weight (N) children by polymerase chain reaction (PCR-RFLP). Body composition was determined by anthropometric method.
Results: The frequency of the Arg/Arg genotype was significantly higher in O compared to N children (O: 36% versus N: 19.8%; $p < 0.001$). In the obese group subject with Arg/Arg genotype had significantly higher body weight (83.5 [22.3] v. 76.4 [19.7] kg, $p < 0.05$), body mass index (BMI) (32.1 [4.8] v. 29.3 [3.5] kg/m^2, $p < 0.001$), body fat % (BF%) (41.3 [3.8] v. 39.5 [4.3], $P < 0.01$), and relative body weight (RBW) (172.8 [23.9] v. 159.6 [20.7] %, $P < 0.001$) as compared to those of Gln/Gln genotype, and significantly higher BMI (32.1 [4.8] v. 30.2 [4.5] kg/m^2, $P < 0.01$) and RBW (172.8 [23.9] v. 163.9 [21.6] %, $P < 0.01$) as compared to those of Gln/Arg genotype. Data are shown as mean and [SD]. There were no significant differences in body composition variables among the three genotypes in normal weight children.
Conclusion: According to our results there is a strong association of the LEPR gene Gln223Arg polymorphism with obesity and among obese children with the degree of obesity.
Funding: This abstract was funded by the Hungarian Ministry of Welfare (113/2003 for D.M and 273/2006 for D.M).

T1:PO.48

Lipoprotein (a) is an atherogenity marker of good prediction – Roman population particularities

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The lypoprotein role in atherogenesis is very important, but unequal. The B phenotype–small and dense LDL (Lp(a)) has a predictive value for cardiovascular disease risk, regardless other lipidic parameters' levels. They can appear in the patient's plasma even if triglycerides are <150mg%.
The aim of the study: The Lp(a) significance in cardiovascular disease evolution for two groups of obese patients: group I – 40 romanian obeses and group II – 39 gipsy obeses. The Lp(a) was performed in the same time with seric lypoproteins' electrophoresis, the working kit being adjusted for the recognition of Lp(a**)**.
Results and discussions: The average level of seric lypoproteins were significantly higher in group II for VLDL (P<0.001) and lower for HDL (P<0.01); LDL were less differentiated (P<0.3). The cardiovascular disease incidence was significantly higher in group II (P<0.001). The average level of Lp(a) is sensibly differentiated in group's II favour (P<0.001). In both groups, cardiovascular disease correlate with heredo-colateral antecedents of cardiovascular disease (arterial hypertension, ischaemic heart disease, cerebral stroke, atherosclerotic arteriopathy) – group I: P<0.0001; group II: P<0.00001). This confirms the genetic component expressed by the presence of Lp(a).
Conclusions: The Lp(a) level is an important marker of cardiovascular diseases' evaluation, regardless other lipidic factors and it is correlated in reverse proportion with LDL. Key words: Lp(a), cardiovascular diseases, obesity.

T1:PO.49

Minor role of brown fat thermogenesis in IL-1beta-induced fever

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The activity of brown adipose tissue (BAT), a site of non-shivering metabolic thermogenesis, has been reported to increase after interleukin (IL)-1ß/lipopolysaccharide injection. To clarify the possible contribution of BAT thermogenesis to whole body febrile response, we investigated febrile and thermogenic response to IL-1ß using mice deficient in uncoupling protein (UCP) 1, a key molecule for BAT thermogenesis. In wild-type (WT) mice, intraperitoneal injection of IL-1ß (5•g/kg) increased plasma corticosterone level and body temperature, and decreased physical activity, and produced a slight and insignificant rise in oxygen consumption (VO_2). VO_2 dependent on metabolic thermogenesis ($\square VO_{2\text{-thermogenesis}}$) calculated by correcting the effect of physical activity was increased after IL-1ß injection (726 ± 200 ml/h/kg at 1 hr). Almost the same responses were observed in UCP1-deficient mice, showing 638 ± 87 ml/h/kg of $\square VO_{2\text{-thermogenesis}}$ at 1 hr. In contrast, intraperitoneal injection of CL316,243 (100g/kg), an activator of BAT thermogenesis, increased body temperature, decreased physical activity, and produced a significant rise in VO_2 in WT mice, showing 1229 ± 35 ml/h/kg of $\square VO_{2\text{-thermogenesis}}$ at 1 hr. Such changes were not obsereved in UCP1-deficient mice, and $\square VO_{2\text{-thermogenesis}}$ at 1 hr was 71 ± 103 ml/h/kg. These results, conflicting with a previously proposed idea of a role of BAT in fever, suggest a minor contribution of BAT thermogenesis to IL-1ß-induced fever. In support of this, we found no effect of IL-1ß on triglyceride content and UCP1 mRNA level in BAT, in contrast with apparent effects of CL316,243.

T1:PO.50

Association of MKKS gene polymorphisms with obesity in Greeks

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Introduction: Obesity is a widely spread multifactorial disease accompanied with a variety of other abnormalities. At present, more than six hundred genes, markers and chromosomal regions are linked with human obese phenotype. The aim of this study was to evaluate the association of seven MKKS gene polymorphisms (SNPs) with obesity in Greeks.
Methods: 220 obese (BMI>30Kg/m^2) individuals and 120 non-obese unrelated subjects (controls) were studied. DNA was extracted from peripheral blood and polymorphisms were analyzed by PCR/RFLPs or sequencing. Mean values of biochemical variables were calculated and compared between carriers and noncarriers of different alleles for each polymorphic site.
Results: The genotype frequencies of two out of the seven polymorphisms were found to differentiate statistically significant obese individuals from the controls. Specifically, the 985+16T>G variation was found mainly in obese individuals (7.85% in obese vs 1.47% in controls, *P*=0.0009) and G allele was found to be associated with an increase in blood pressure. Moreover, a new polymorphism (1129C>T) was detected exclusively in obese subjects (9.12%) and T allele was associated with significantly high HbA1c values (6.84±4.87 mg/dl in carriers vs 5.67±1.23 mg/dl in non-carriers, *P*=0.037). Twenty-six composite genotypes resulted from the combination of the seven SNPs. Two of them were found to be associated with the obese phenotype.
Conclusions: This study suggests that MKKS gene SNPs are associated with genetic predisposition to obesity in Greeks. The understanding of the genetic component of obesity in combination with daily exercise and appropriate nutrition would be an effective strategy to prevent obesity.

T1:PO.51

The Nutrient - Induced Insulin Output Ratio (NIOR) as a possible biomarker of fat or carbohydrate susceptible phenotype

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Background: Metabolic regulation, from genes to metabolites, dictates biochemical functions as well as the nutritional and dietary demand. Therefore, genetic disposition and metabolic needs are important in determining the optimal diet for an individual in prevention of metabolic syndrome.
Methods: The insulin output as area under insulin curve after glucose tolerance test (AUCIns OGTT) and lipid tolerance test (AUCIns OLTT) were measured within 167 members of the study group. Estimation of the 18 common of "obesity risk-genes" polymorphisms and standard phenotyping was performed.
Results: Insulin output during oral glucose tolerance test (AUCIns OGTT) correlated strongly with insulin output after standard high fat meal (AUCIns OLTT) in the whole group. However, within the genotypic sub-groups the correlation was lower or even does not exist. The new insulin index NIOR which is the product of the division of area under insulin curve (AucIns) obtained during oral lipid tolerance test (OLTT) and area under insulin curve (AucIns) obtained during standard oral glucose tolerance test (OGTT) may be used to validate the genetic markers in the aspects of food susceptibility. The mean value of NIOR index is 1,66 [value varied from 0,42 to 5,83 within individuals] and is dependent of BMI, but not dependent on gender, WHR, HOMA or adiponectin level. The high NIOR value pointed to the worse tolerability of lipids than of glucose content in the meal. The NIOR index seems to have the predictive value for discrimination between the phenotype susceptible for adipogenic activity of fat or carbohydrate containing diet.
The identification of common genetic variants connected with increased risk of high or low NIOR may optimize the use of medical resources through early identification of subpopulations at risk and targets groups for early prevention. It is the new way to evaluate early genetic biomarkers (genetic variants of genes involved in development of obesity and insulin resistance) based on whole body insulin response to different type of diet.
Funding: Granted by Polish MNiI: Nr 3PO5D08424 and Nr 501/NKL/49/L and NuGO, a Network of Excellence funded by the European Union (CT-2004–505944).

T1:PO.53

The influence of neuropeptide SF on the lipolytic capacity of human preadipocytes

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Introduction: Recently it has been shown that the neuropeptide receptor, NPFFR2, known to be involved in pain modulation is expressed in human adipose tissue and that this receptor affects adipocyte b-adenoceptor signalling in the 3T3-L1 mouse fat cell line. NPFFR2 is closely related to neuropeptide receptor NPFFR1. In the current study we tested whether NPFFR1 may be involved in the process of catecholamine-induced adipocyte lipolysis. For this purpose we tested the effects of the natural ligand of NPFFR1, NPSF, on human fat cells *in vitro*.
Subjects and Methods: Human subcutaneous adipose tissue was obtained from healthy women undergoing abdominal liposuctions for cosmetic reasons. The differentiated preadipocytes were treated with a maximum effective concentration of NPSF (1μM) for 3 or 48 hours prior to liopolysis experiments.
Results: Three or 48 hours pretreatment of differentiated preadipocytes with NPSF significantly reduced noradrenaline-induced lipolytic capacity both in the presence or absence of the a2-adrenoceptor antagonist yohimbine demonstrating an interaction with the b-adrenoceptor signalling pathway. However, NPSF had a significantly higher reducing effect on postreceptor induced lipolysis as demonstrated by the markedly reduced lipolytic effect of dibutyryl cyclic AMP, which acts on the protein kinase A-hormone sensitive lipase complex.
Conclusion: NPFFR1 is involved in the regulation of human fat cell lipolysis. Stimulation with the natural ligand, NPSF, resulted in inhibition of catecholamine-induced lipolysis, which may be attributed to interactions between b-adrenoceptors and NPFFR1 signalling at a distal level (i.e. at the level of the protein kinase A-hormone sensitive lipase complex).

T1:PO.54

Varying Effects of Flavanols on Adipokine Release from 3T3-L1 Adipocytes

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Background: Flavanols are reported to improve endothelial function, and reduce adipose tissue mass *in vivo*. This study compares the effects of two flavanols (catechin and epicatechin) on adipokine secretion from cultured adipocytes.
Methods: 3T3-L1 preadipocytes were seeded in 35mm dishes. Two days after reaching confluence differentiation was induced using 1 -methyl-3-isobutylxanthine, dexamethasone and insulin. Gr oups of six dishes were exposed to vehicle, 1, 5 and 10 μM (+)-catechin hydrate (Cat) or (-)-epicatechin (Epi) for 6, 12 and 24hr. Media was then removed and assayed for adiponectin, leptin and resistin using ELISAs.
Results: Treatment with 1 and 5μM Cat caused a stepwise reduction in resistin release, whereas treatment with 10μM Cat significantly increased resist in release compared to 1 and 5µM (P<0.001). A similar pattern was observed for Epi, with 10μM Epi producing significantly higher resistin release than 1 and 5µM (P<0.01). A time -dependent effect was seen with 10µM Cat and Ep i, with higher resistin release at 24hr compared to 12hr (P<0.001). Treatment with Epi for 24hr caused a dose -dependent stepwise increase in adiponectin release, with 10μM Epi ~ 2 fold higher compared to vehicle (P<0.001). Cat appeared to decrease adiponectin release, with 5µM giving a 38% decrease compared to vehicle. Leptin release increased 1.5 fold with 1μM Epi and 2.9 fold with 5μM Epi (P<0.01) compared to vehicle. Cat treatment did not affect leptin release.
Conclusions: The flavanols, Cat and Epi, had differential effects o n adipokine release, the mechanisms of which remain to be determined.
Research relating to this abstract was funded by the BBSRC.

T1:PO.55

Serum Amyloid A1 induces insulin resistance and inflammation in human adipocytes

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In human obesity, white adipose tissue plays a predominant role in the low grade inflammatory status and contributes significantly to the production of cytokines. Human adipocytes have recently been shown to secrete acute phase proteins like Serum Amyloid A1 (SAA1). SAA1 is a major acute phase protein, previously thought to be secreted exclusively by the liver. Acute phase response is characterized by major metabolic changes aimed at mobilizing energetic stores including increased adipose tissue lipolysis. In order to better understand the contribution of SAA1 in these changes, we have treated human Multipotent Adipose Derived Stem (hMADS) cells differenciated in adipocyteswith recombinant SAA1.
Methods: Triglycerides content was quantified using AdipoRed. Gene expression was measured through quantitative RT-PCR. Lipolysis was quantified through glycerol release. Adiponectin and MCP-1 releases were measured by ELISA. Insulin stimulated glucose uptake was measured using [3H] 2-deoxyglucose. Electrophoretic mobility shift assay (EMSA) was performed to follow NFB activation.
Results: recombinant SAA1 (0.5 – 5 μg/mL) was incubated with differenciated hMADS cells for 30 min to 3 days depending on the readouts. SAA1 dose-dependently inhibited triglycerides accumulation and adiponectin secretion. SAA1 stimulated basal lipolysis and MCP-1 secretion. SAA1-treated adipocytes showed decreased insulin stimulated glucose uptake. SAA1 gene expression revealed down-regulation of adipogenic genes such as PPARg2 and C/EBP while showing up-regulation of NFB target genes such as MCP-1, SAA1, Haptoglobin and PAI-1. SAA1 induces the translocation of the NFB complex as shown by EMSA.
Conclusion: Our study suggests that SAA1 directly induces lipolysis while inhibiting lipogenesis and insulin stimulated glucose uptake in human adipocytes. SAA1 plays a major role in the mobilization of adipocyte triglycerides during the acute phase response. Activation of the NFkB pathway by SAA1 also induces inflammation in human adipocytes.

T1:PO.56

Lipoprotein lipase gene polymorphism, /lipemia postprandial glicaemia and obesity

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Background: Genetic variants of the lipoprotein lipase are associated with obesity, dyslipidemia, and insulin resistance. Aim of the study was to investigate whether LPL-HIND III of lipoprotein lipase gene polymorphism is linked to changes in postprandial metabolic status paramethers in patients with familial obesity.
Methods: 163 representatives of 40 obese families underwent clinical and laboratory metabolic assessment. Blood lipids (cholesterol, TG anf FFA), insulin, glucose, leptin, adiponectin and leptin/adiponectin ratio (lep/adip) were determined after 12 h fasting and during oral glucose (OGTT) and lipid (OLTT) tolerance tests. The polymorphism of
lipoprotein lipase T->G in intron 8 (LPL-HIND III) was measured by PCR-RFLP.
Results: Patients with LPL-H vs8 GG genotype had significantly higher body weight (P<0,05) and WHR (P<0,05) and the lowest HDL-chol. level (P<0,001) compared to LPL-H vs8 TG and LPL-H vs8 TT genotypes. LPL-H vs8 GG subjects demonstrated the highest TG and FFA blood concentration (P<0,05) during OLTT, as well as insulin output measured as the 2 h insulin blood level during OLTT (P<0,05). The highest mean leptin concentration (20,55 mg/l) was detected in group with LPL-H vs 8 TG genotype. No differences in adiponectin level was observed between various genotypes, but the lep/adip ratio was the highest in LPL-H vs 8 TG genotype. No difference in postprandial glucose level among studied genotypes was detected (OGTT).
Conclusions: These results suggest, that LPL-H vs8 GG polymorphism may play a role in the development of metabolic complications of obesity.
Supported by: Polish grants: 2 P05A 067 28, 501/NKL/48/L, 501/KL/559/L.

T1:PO.57

Retinol Binding Protein 4 in obesity models and its regulation in adipocytes by retinoic acid

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Introduction: Retinol Binding Protein 4 (RBP4) is synthesized in liver and adipose tissue. RBP4 serves to transport retinol from tissue storage sites to vitamin A –dependent tissues. Mice with defects in insulin action showed increased levels of rbp4 mRNA in adipose tissue and serum RBP4. Thus, alterations of adipocyte-derived RBP4 could influence insulin action. Retinoic acid (RA) treatment improves glucose tolerance and decreases the expression of resistin (an adipokine that impairs insulin action) in mice. Accordingly, RA may act on RBP4 production.
Aim: To check serum RBP4 levels in rodent obesity models and RBP4 regulation by RA in murine adipocytes *in vitro* and *in vivo*.
Materials and Methods: Serum RBP4 levels were determined by western blot in obese and lean Zucker rats, and in mice (NMRI and B6) fed a normal -fat diet (NF) or a high - fat diet (HF). RBP4 mRNA was determined by qPCR in RA -treated 3T3-L1 adipocytes, and adipose depots of RA-treated NMRI mice and NMRI mice fed NF or HF diet.
Results: HF diet did not modify serum RBP4 levels in B6 or NMRI mice, and reduced adipose rbp4 mRNA in the latter. Serum RBP4 levels were higher in lean versus obese Zucker rats. RA treatment down-regulated rbp4 mRNA in 3T3 -L1 adipocytes and in adipose depots and liver of NMRI mice. However, serum RBP4 levels were increased after RA-treatment.
Discussion: According to our d ata, high circulating RBP4 levels are not extensible to all insulin-resistant and obesity models. RA exert s distinct effects on rbp4 transcription and RBP4 levels in serum.

T1:PO.58

Apo E polymorphism, postprandial lipemia, leptin to adiponectin ratio, and insulin sensitivity in obese families

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Apolipoprotein E (apo E) polymorphism may be a risk determinant of coronary heart disease (CHD), since the apo E gene polymorphism is associated with increased fasting and postprandial levels of triglycerides. The study on apoE deficient mouse documented that the elevated plasma adiponectin suppresses the development of atherosclerosis *in vivo*.
Methods: Apo E phenotyping was performed by isoelectrofocusing method of 281 members of familial obesity group (104 men and 177 postmenopausal women). The fasting lipids, adiponectin and leptin as well as during postprandial lipemia test the insulin and triglicerides levels up to 8 hours were measured. The HOMA index as calculated.
Results: The E3/3 genotype occurred in 73.5% of the patients, followed by E3/4, E2/3, E2/4, E2/2 and E4/4 genotypes, which occurred in 15.4%, 9.6%, 0.5%, 0.5% and 0.5% of the patients, respectively. The significant difference in adiponectin and leptin concentration were observed between the group carrying the apolipoprotein epsilon4 and epsilon 2 allele. The lowest adiponectin and highest leptin concentration were characteristic for the epsilon2 allele apoE carriers. The value of leptin to adiponectin ratio was also the highest in this group. The insulin resistance index HOMA-IR was the highest in the epsilon 2 allele carriers group (the statistical significance (P=0,007) between apo E3/3 genotype and epsilon 2 allele carriers).
Conclusion. The association of common polymorphism of apolipoprotein E and postprandial lipemia and the adipocytokine level argue for causative link between the risk of obesity and atherosclerosis. *This work was supported by Ministry of Informatics and Science grants 4P05D08424; 2P0A 06728, 501/NKLl/49/L, 501/NKLl/48/L.*

T1:PO.59

Modulation of adipose visfatin expression by retinoic acid and rosiglitazone

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Introduction: Visfatin was recently identified as an insulin –mimetic produced by white adipose tissue (WAT). Visfatin may have paracrine/autocrine effects facilitating differentiation and fat deposition in adipocytes. However, controversy exists as to whether visfatin is up –regulated or not by PPAR g agonists known to favour WAT expansion, such as rosiglitazone. Retinoic acid (RA) is a fat mobilising agent that influences the express ion of several adipokines.
Aim: To check the modulation of visfatin expression by RA and rosiglitazone in cultured murine adipocytes, and by RA *in vivo*.
Material and Methods: Visfatin mRNA (qPCR) and protein levels (western blot) were analysed in 3T3 -L1 adipocytes treated with RA and rosiglitazone, and in WAT and serum of RA-treated NMRI mice.
Results: In 3T3-L1 adipocytes, 9 -cis-RA (1 mM) and all-trans-RA (1 mM) triggered a late up-regulation of visfatin mRNA (45% increase after 48 and 72 h treatment) wh ich was preceded by a down -regulation (50% reduction after 36 h treatment). Cellular visfatin protein levels followed similar changes. 1 mM rosiglitazone treatment for 24, 48 and 72 h up -regulated visfatin mRNA levels in 3T3 -L1 adipocytes. In mice, all -trans-RA treatment reduced visfatin mRNA in WAT depots, but did not affect circulating visfatin.
Discussion: Up-regulation of adipose visfatin may contribute to the p ro-adipogenic effect of rosiglitazone. Down -regulation of adipose visfatin may contribut e to the antiadipogenic effect of RA. Late -onset up -regulation of visfatin by RA seen in 3T3 -L1 adipocytes may be part of a ne gative feed-back loop to avoid fat depletion, which however is lacking in the *in vivo* setting used.

T1:PO.60

Retroperitoneal adipose tissue in ferret (Mustela putorius furo) has a higher oxidative capacity than the interscapular adipose depot

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Brown adipose tissue, characterized by a high mitochondrial capacity, is primarily localized in the interscapular region in rodents, although certain species, as man and lamb, have a tissue that resembles the brown depot, but have it located mostly perirenally. Here we have studied the oxidative capacity in different adipose tissue depots of ferrets at different temperatures. For this purpose we have measured the cytochrome coxidase (COX) activity as an index of the aerobic-oxidative mitochondrial capacity in the retroperitoneal, inguinal and interscapular adipose tissue of two groups of adult ferrets, one housed at 22ºC and other acclimated to 4ºC for one week. Protein and COX activity were measured in the initial tissue homogenate and in the final mitochondrial suspension. Our data show a higher COX activity in the retroperitoneal adipose depot in comparison to the inguinal and the interscapular ones, when considering both the specific-activity (per mg of protein) and the total activity (per g of tissue), and both in the initial tissue homogenate and in the final mitochondrial suspension. In fact, contrary to what happens in rodents, the interscapular adipose tissue showed the lowest levels of enzymatic activity. No important change due to cold acclimation was observed in none of the adipose depots studied. We can conclude that the retroperitoneal adipose tissue of ferrets resembles brown adipose tissue found in other species in terms of oxidative capacity.

T1:PO.61

The effect of red grape seed on lipid serum levels of hypercholesterolemia subjects

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Background: Cardiovascular diseases (CVD) are of the most important causes of mortality in the world, and it has been estimated 38.5% in Iran. Dietary factors have significant influence on the control and preventive effects on CVD and lowering lipid serum levels. This study was conducted to evaluate the effects of red grape seed on the level of total cholesterol, LDL-C, HDL-C, TG and FBS of hypercholesterolemia women.
Materials & Methods: In a clinical trial 35 hypercholesterolemia women were randomly selected in 2 groups of red (RG) and control (CG), RG included 18 subjects with 49 years old and BMI 27, CG included 17 subjects with 52 years old and BMI 28, RG group was fed 500gr red grape seed per day for 4 weeks. Control group was without any treatment. At the beginning of the study, physical activity, height, weight and BMI were determined. Lipids serum levels (TC, LDL-C, HDL-C, TG and FBS) were measured for 2 times (beginning, after 4 weeks). Dietary intake was also determined for 6 days during the study by using 24hrs recall method. Experimental findings were analyzed statistically by suing T-test and paired t-test in software "SPSS". The amount of crude fiber and flavonoid were measured .
Results: The mean levels of TC in RG group significantly decreased (-25.5 mg/dl) at the end of the 4 weeks experiment (P=0.027), also shows significance with CG (P=0.01). LDL-C level decreased significantly in RG group (-22.3 mg/dl) at the end of 4 weeks (P=0.001) and decreased significantly with CG(0.008). HDL-C, TG and FBS differences were not significant at the end of the experiment.
Conclusion: Regarding the findings whole red grape seed would cause reduction of TC and LDL-C. Therefore, consumption of grapes with seeds and skin containing flavonoid and fibers were recommended to promote health.

T1:PO.62

Metabolism in adipose tissue in response to Citalopram and Trimipramine treatment - an *in situ* microdialysis study

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The intake of antidepressants is often accompanied by weight gain. Antidepressants may influence lipid and carbohydrate metabolism that can result in metabolic changes and obesity. The aim of the study was to investigate the effect of antidepressants on metabolism of subcutaneous adipose tissue. Subjects: Obese subjects on treatment with citalopram and trimipramine, respectively, were compared to matched controls. Each group comprised 10 subjects. Methods: Interstitial concentrations of glycerol, glucose and lactate and local blood flow were measured in subcutaneous adipose tissue by means of the microdialysis technique. Glycerol was analyzed by bioluminescence, glucose and lactate were measured electrochemically. Blood flow was assessed by the ethanol escape technique with ethanol measured by gas-chromatography. Results: Under basal condition glucose and lactate concentration were elevated in subjects treated with citalopram. Following perfusion (2.5 μl/min) with norepinephrine (10^{-4} M) glycerol concentration was prolonged in subjects under anti-depressant treatment. Adipose tissue glucose concentration decreased, but showed no difference to controls. Likewise, lactate concentration increased, without any difference to controls. Adipose tissue blood flow decreased in control groups due to norepinephrine application, but remained constant in antidepressant groups. In conclusion citalopram and trimipramine affect activity of norepinephrine and resulted in enhanced glycerol release into the circulation. Antidepressant therapy may contribute to weight gain through amplified activity of the sympathetic nervous system.

T1:PO.63

The G241R adhesion molecule-1 (ICAM-1) gene polymorphism is associated with||obesity in the Italian population

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Objective: Two polymorphic variants have been described in the human ICAM-1 gene and have been shown to be significantly associated with several pathologies of inflammatory origin. In this study we evaluated whether these ICAM-1 gene variants are associated with obesity and can influence its protein levels.
Methods: Allele-specific PCR and RLFP were used to investigate the presence of G241R and E469K polymorphisms in 237 severely obese subjects (BMI 43±7.2 Kg/m^2). One hundred fifty two normal weight individuals (BMI 22.4±2.7 Kg/m^2) were also screened as a control group.
Results: The G241R genotype distribution was significantly different between obese and control group (P=0.003). Moreover, the frequency of the R allele was 2-fold higher in obese patients compared to control subjects (0.085 vs 0.033, P=0.004; OR, 2.7; 95% CI, 1.3-5.5). When sICAM-1 levels were evaluated according to the G241R genotype, no significant differences were observed. However, as already reported, significant higher sICAM-1 levels were detected in the obese subjects. Finally the allelic frequency of the E469K polymorphism was not different between obese and normal weight subjects.
Conclusions: To the best of our knowledge, this is the first evidence of an association between the ICAM-1 gene G241R variant and obesity. As this polymorphism does not seem to influence sICAM-1 levels, we can speculate that the presence of the R allele might have a role in the recruitment and activation of immune cells and, thus, might contribute to the low grade inflammatory state present in obese subjects.

T1:PO.64

The CD34+/CD31- progenitor cells of the human adipose tissue: Local control of their proliferation and fate

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The stroma-vascular fraction of adipose tissue (AT) contains capillary endothelial cells (CECs), inflammatory cells and CD34+/CD31- cells that are able, under appropriate culture conditions, to differentiate into adipocytes or CECs. The present study was performed to analyse the local stimuli involved in the growth and fate of the CD34+/CD31- cells. Mature adipocytes, CECs, CD34+/CD31- cells were isolated from the human subcutaneous AT by an immunoselection/depletion approach as previously described. Conditioned media from freshly harvested adipocytes and CECs were collected. The growth and migratory responsiveness of the CD34+/CD31- cells was determined by BrdU incorporation and Boyden chamber assays, respectively. The CD34+/CD31- differentiation was assessed by the expression of specific adipocyte and CECs markers. CD34+/CD31- cells expressed receptors for adipokines, leptin and adiponectin, growth factors and chemokines. Adipocyte- and CECs-conditioned media induced the proliferation and the migration of the CD34+/CD31- cells. Whereas adipokines had no proliferative effect, several inflammatory cytokines as well as growth factors stimulated the growth of the CD34+/CD31- cells. Migration of CD34+/CD31- cells to conditioned media involved a chemotaxis response mediated through the activation of Gi-coupled receptors. Finally, a medium allowing the differentiation of the CD34+/CD31- cells into adipocytes and CECs was defined. To conclude, the present study strongly suggests that stimuli arising from mature adipocytes and CECs modulate the growth and differentiation of the AT-derived CD34+/CD31- cells. Such local signals might be involved in the control of adipogenesis and vascular network extension and thus in the fat mass development with obesity.

T1:PO.65

Influence of sex hormones on adiponectin expression in human adipocytes

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Introduction: Adiponectin is an adipocytokine with profound anti-diabetic and anti-atherogenic effects. Plasma adiponectin concentrations are significantly higher in women than in men. In order to study the molecular aspects of this gender specific dimorphism, we examined the expression of adiponectin under the influence of sex hormones.
Methods: As a model system we have used human SGBS preadipocytes and adipocytes. Differentiating cells (d 8) as well as mature adipocytes (d 14) were incubated with increasing doses of testosterone (1 – 1000 ng/ml) or estradiol (0,1 – 100 nM). Adiponectin mRNA expression was determined by RT-PCR using specific primers. Adiponectin secretion into the medium supernatant was measured by ELISA.
Results: Adiponectin mRNA expression was up-regulated during adipogenic differentiation. Adiponectin secretion into the culture medium was detected starting at d 7 (~ 50 ng/ml). Neither testosterone nor estradiol treatment had an influence on adiponectin mRNA expression in human adipocytes. Adiponectin expression was unaffected even after long incubation periods for up to 7 days. Preliminary data show that secretion of adiponectin was constant upon treatment with both sex hormones. Further experiments using BMImatched male and female serum samples will provide new insights into regulation of adiponectin expression by systemic humoral factors.
Conclusion: Our data suggest that testosterone and estradiol have no direct influence on the expression of adiponectin mRNA in SGBS adipocytes. Thus, we hypothesize the existence of a systemic factor which is regulated by sex hormones and subsequently causes the sexual dimorphism in adiponectin plasma levels.
Research relating to this abstract was funded by the Landesforschungsschwerpunkt “Metabolisches Syndrom”, Universität Ulm.

T1:PO.66

Regulation of MCP-1 and IL-6 expression by NFkB in human adipocytes

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Obesity is a state of chronic low -grade inflammation associated with elevated plasma levels of proinflammatory cytokines (e.g. TNF -α, IL-6), chemokines (e.g. MCP -1) and prothrombotic proteins (e.g. PAI -1), which are in volved in development of obesityrelated diseases such as type 2 diabetes and atherosclerosis. Adipose tissue and in particular also adipocytes are known to produce these so called adipokines, among them the monocyte-chemoattractant protein -1 (MCP-1). Recent transgenic mouse data implicated MCP-1 in mediating adipo se tissue macrophage infiltration, which may additionally promote the proinflammatory state in obese rodents and humans. In this study, we analysed the regulation of MCP -1 expression in human adipocytes. The human preadipocyte cell line SGBS was stably infected with different proteins interfering dominant negative ly or active ly with the NFkB signalling pathway. Transient transfection of the cells with luciferase reporter constructs, regulated by MCP -1 and IL-6 upstream regulatory sequences w as performed. Furthermore, the regulation of the endogenous genes in stable cells w as analysed by qRT -PCR, and protein secretion into the cell culture medium by ELISA. It could be show n that the MCP -1 and IL-6 gene are regulated by NFkB in human preadipocytes both at the basal expression level and upon TNF-α stimulation. Using this approach, we are currently further analys ing in detail which components of the NFkB pathway are involved in adipokine regulation in human preadipocytes and adipocytes. In conclusion, our data suggest that the NF kB pathway plays a central role in the regulation of the proinflammatory proteins MCP -1 and IL-6 in human adipose tissue.

T1:PO.67

Down-regulation of cytokines and adipokines linked to inflammation and insulin resistance by atrial natriuretic peptide in human adipose tissue

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Increased adipose tissue (AT) secretion of adipokines and cytokines has been involved in the chronic low-grade inflammation state and insulin resistance associated with obesity. We tested here whether the cardiovascular and metabolic hormone ANP (atrial natriuretic peptide) was able to modulate AT secretion of several adipokines (derived from adipocytes) and cytokines (derived from AT stromal cells) linked to insulin resistance either directly or through induction of lipolysis. We used protein array to measure the secretion of adipokines and cytokines after 24h culture of human AT explants treated or not with a physiological concentration of ANP. The gene expression profile of the regulated factors was determined by RT-qPCR on total RNA from adipocytes obtained after collagenase digestion and macrophages isolated from the stromavascular fraction by CD14- positive immunoselection.
ANP decreased the secretion of the pro-inflammatory cytokines interleukin-6 and tumor necrosis factor- , of many chemokines, and of the adipokine leptin and retinol binding protein 4 (RBP-4). The secretion of the anti-inflammatory molecules interleukin-10 and adiponectin remained unaffected. The cytokines were mainly expressed in macrophages that expressed all components of the ANP-dependent signaling pathway. The adipokines, leptin, adiponectin and RBP- 4 were specifically expressed in mature adipocytes. The inhibitory effects of ANP on leptin and growth related oncogene secretions were neutralized under selective hormone sensitive lipase inhibition. These data suggest that ANP down-regulates the secretion of several adipokines, chemokines and cytokines with a role evoked in insulin resistance in humans either directly or through induction of lipolysis.

T1:PO.68

Pattern of expression of adiponectin receptors in the liver and its relation to non-alcoholic steatohepatitis

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In mice with non -alcoholic-steatohepatitis(NASH), adiponectin(APM1) and its receptors are thought to mediate anti -steatosis/anti-inflammatory effects. Our aim was to define APM1 expression in subcutaneous -SAT- and visceral -VAT- adipose tissue and expression of its receptors in the liver (LAPMR1/LAPMR2) in relation to liver histology in morbidly obese patients. Total-RNA was extracted from SAT, VAT and liver tissues obtained during gast ric-bypass from 71 subjects (BMI 47.1±7.6 kg/m^2). Relative quantifications of *APM1*,*LAPMR1*,*LAPMR2* was performed by Real -Time-PCR. Subjects were divided according to liver histology: 7 normal liver[NH]; 43 with steatosis: 23 with NASH. Results were expressed as the target/HPRT ratio. Plasma adiponectin levels were higher in NH than NASH (5.3±2.0 *vs* 4.8±2.9 *vs* 3.4±1.2µg/ml, respectively, P=0.05). VAT-APM1 was significantly more expressed in NH than NASH (0.74±0.26 *vs* 0.70±0.53 *vs* 0.48±0.30, P=0.05). Plama adip onectin was directly related to VAT -APM1 expression (r=0.41, P<0.004). In contrast, LAPMR1 was less expressed in NH than NASH (1.05±0.14 *vs* 1.26±0.33 and 1.44±0.40, $P \leq 0.02$) as was LAPMR2 (0.78±0.28 *vs* 0.97±0.39 and 1.23±0.47, $P \leq 0.02$). In multivariate model s adjusting for sex, age, and BMI, liver enzymes (AST,ALT,gGT) levels were related to LAPMR1(*s.d.r*=-0.43,P<0.0005;*sd.r*=-0.38,P<0.003;*s.d.r*=-0.32,P<0.02) and LAPMR2 expression (*sd.r*=-0.31,P<0.01;*sd.r*=- 0.30,P<0.02;*sd.r*=-0.45,P<0.0002). Furthermore, LAPMR1 and LAPMR2 expression were reciprocally related to plasma adiponectin (r=0.46,P=0.0016 and r=0.30,P=0.05, respectively). We conclude that in severely obese subjects NASH is associated with a lower adiponectin levels in plasma and expression in VAT, but with higher expression of its receptors in the liver. The pattern of association suggests that hepatic adiponectin receptors expression is upregulated in the presence of NASH as a compensatory response to hypoadiponectinemia.

T1:PO.69

Pathogenetic factors in obesity contributing to the development of heart failure

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As a consequence of obesity-related haemodynamic, metabolic and neuroendocrin alterations, left ventricular hypertrophy, diastolic and systolic dysfunction occurs, finally overt congestive heart failure with low ejection fraction develops. The possible correlation of anthropometric and metabolic parameters with structural and functional alterations on echocardiography was studied in 50 obese patients (age:40.65±11 yrs, BMI:39.27±7.36 kg/m^2), and 42 non-obese age-and sex-matched normal controls (age:37.29±10.09 yrs, BMI:22.27±1.81 kg/m^2). Blood samples were assayed for "routine" laboratory parameters and oxLDL, TBARS, FFA, leptin, adiponectin, resistin, sE-selectin, PON1-activity, NO, TNFα, and IL-6. Correlations between variables were assessed by Spearman correlation. Significant ($P \leq 0.05$) correlation was found between adiponectin and the following parameters: HDL-C (r=0.56), ApoA-I (r=0.43), PON1-activity (r=0.23), waist circumference (r=-0.37), insulin (r=-0.40), triglyceride (r=- 0.46), and sE-selectin (r =-0.23). sE-selectin correlated with HDL-C (r=-0.33), triglyceride (r=0.25), and diastolic blood pressure (r=0.28). BMI correlated significantly with insulin (r=0.52), HOMAR (r=0.41), HgbA1C (r=0.42), C-peptide (r=0.57), adiponectin (r=-0.49), leptin (r=0.79), and resistin (r=-0.26). Left ventricular mass index correlated significantly with BMI (r=0.39), waist circumference (r=0.49), HgbA1C (r=0.55). Ejection fraction correlated significantly with HDL-C (r=0.33), C-peptide (r=-0.36), and HgbA1C (r=-0.38). Systolic peak velocity of the myocardium was significantly lower in each segment measured by myocardial Doppler imaging in obese patients compared to non-obese subjects. Transmitral E/A and myocardial Doppler imaging data indicated an initial relaxation problem in obesity. In conclusion an early target organ damage can be detected in obese patients, and a correlation exists between metabolic alterations and changes in structural and functional echocardiographic parameters.

T1:PO.70

Association between endothelial nitric oxide synthase haplotypes, abdominal obesity, and metabolic syndrome

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Metabolic syndrome (MS), a cluster of several metabolic disorders, is increasingly recognized as a risk factor for cardiovascular disease. Data from animal models indicate that the endothelial nitric oxide synthase gene (eNOS) null mice present with a phenotype of insulin resistance, hypertension and hypertriglyceridemia, much like that observed in humans with MS. Endothelium-derived nitric oxide facilitates skeletal muscle glucose uptake. Here, we investigate the role of genetic variation in eNOS as assessed by haplotype tagging single nucleotide polymorphisms (htSNPs) with abdominal obesity and other features of MS. Altogether, 738 unrelated subjects were recruited from a cross-sectional populationbased epidemiological survey in the province of Segovia in Central Spain (Castille). BMI, waist circumference. Fasting and 2 hrs glucose, serum insulin, leptin, adiponectin levels; lipid profile. MS was defined according to the recently modified ATP III guidelines. Haplotype analysis showed that there is a statistically significant association between the eNOS gene variants and MS. Relative to the most common haplotype (121), the haplotype (212) was associated with increased metabolic syndrome risk (OR=1.81, 95% CI 1.15–2.84), decreased HDL-cholesterol (OR=1.52, 95% CI 1.01–2.29), increased HOMA (*P*=0.04), and triglycerides (*P*=0.03) mean values. Furthermore the 222 haplotype was associated with increase abdominal obesity (OR=2.26, 95% CI 1.23– 4.14). Our results suggest that genetic variation at the eNOS locus is associated with features of MS, and might represent a new genetic susceptibility component for abdominal obesity, insulin resistance and hypertriglyceridemia.

T1:PO.71

Crucial role of adipose CoQ content in obesity

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Coenzyme Q (CoQ) is an obligatory partner of mitochondrial biology and also the unique lipophilic antioxidant synthesized in humans. Although involved in numerous disorders, no clear link with adipocyte biology has been yet established. Here, we demonstrate that CoQ is specifically depleted in adipose tissue of obese mice and humans. Co-treatment of obese mice with a PPARg agonist and CoQ fully restored adipose tissue CoQ content. Moreover, CoQ treatment not only counteracted adverse side effects classically induced by PPARg ligands but also improved their effects on energy homeostasis. In addition, CoQ content in adipocytes is positively correlated to adiponectin gene expression, a critical regulator of insulin sensitivity, and associated to decreased TNFa gene expression, and thus to a decreased inflammatory state. In vitro, CoQ modulated PPARg activity in an adipose cell and receptor specific manner through SIRT1. As a whole, these data strongly suggest that adipose CoQ behaves as a key factor of adipocyte biology and the development of obesity.

T1:PO.72

Functional analysis of melanocortin-4-receptor (MC4R) mutants: critical role of W174C substitution in the fourth transmembrane domain

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Introduction: MC4R is involved in obesity and mutations in this receptor account for 4 - 5% of all cases of gene tic obesity. We recently genotyped 200 unrelated obese adults (BMI • 40) from Campania (1). We identified 4 novel mutations: two missense mutation s (W174C and I317V) in the IV transmembrane domain and in the C -tail respectively, and two nonsense mutations (Q43X and S19fsX51) resulting in truncated proteins. Here we report the *in vitro* functional analysis of these muta tions.
Methods: MC4R cDNA wild type (wt) and the W174C and I317V mutants were cloned in a pcDNA 3.1/His vector. Receptor activity was assayed by transient transfection of these plasmids in COS -7 cells with 0.1, 1, 10 and 1000 nM of the natural ligand, a-MSH. We identified the membrane localization of wt and mutant receptors in HEK -293 cells using immunofluorescence staining. Competitive binding for receptor/ligand was evaluated by FACS (2).
Results: Mutants Q43X and S19fsX51 were inactive, as reported for other truncated proteins. The cell-surface expression of mutant I317V was much lower versus wt MC4R. Mutant W174C has no activity, which is surprising given its proximity to the mutant A175T that exerts partial activity. However, its cell - surface expression resembles that of wt receptor. Mutant W174C had reduced a-MSH affinity (50% *vs* 100% wt receptor; Scatchard plot), which could explain its reduced activity. Acknowledgement: Work supported by grants from the Regione Campania (Convenzione 11/06/2003) and MIUR (PRIN 2002).
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T1:PO.73

Differential magnitude in the early response of key genes to short-term fasting in soleus and gastrocnemius skeletal muscles

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Fasting induces metabolic and gene-expression changes in skeletal muscle, and a short term food-deprivation model can help us to uncover the earliest response of key genes. Peroxisome proliferator-activator receptor gamma coactivator-1 alpha (PGC-1α) induces and coordinates gene expression stimulating events as mitochondrial biogenesis, fibre type switching and specific type I fibre gene expression. Uncoupling protein 3 (UCP3) has been involved in limiting ROS formation and the handling of lipids as fuel substrate. We aimed to characterize the response of PGC-1α and UCP3 mRNA expression under short-term fasting in a mixed fibre type muscle (gastrocnemius) with a high capacity to shift between glucose and lipids as fuel substrate and in a muscle rich in slow twitch oxidative fibres (soleus). Five different feeding conditions were studied in adult male Wistar rats: *ad libitum* feeding (control group), 4, 8 and 24-hour fasting, and 3-hour re-feeding after 8- hour fasting (re-fed group). PGC-1α and UCP3 mRNA levels were analysed by RTqPCR. As expected, fasting caused a significant increase in UCP3 mRNA levels in muscle, with a more pronounced rise in the gastrocnemius than in the soleus, returning to control levels in the re-fed group. PGC-1α mRNA expression tended to be increased after 4-hour fasting, also more markedly and significantly in the gastrocnemius, afterwards returning to control levels in both muscles at 8-hour fasting. The results show that *pgc-1* and *ucp3* are early response genes to fasting in skeletal muscle, being its response related to its role in the metabolic shift from glucose to lipid use as fuel.

T1:PO.74

Tissue specific modulation of AMPK phosphorylation in high-fat diet-fed rats treated with 3,5-diiodothyronine (T2) is related to fatty acid demand

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3,5-diiodo-L-thyronine (T2) is able to prevent body weight-gain and fat accumulation when administered to rats receiving a high -fat diet (HFD). It induces an increase in fatty acid (FA) oxidation concomitant to their less efficient utilization. The effects of T2 on fatty acid oxidation involve the AMP-activated protein kinase (AMPK), but it is not clear how its modulation is achieved in tissues such as liver and white adipose tissue (WAT). Six groups of rats were used, either receiving a 1 -week HFD or a 4-week HFD, with or without a daily i.p. injection of T2 (25 mg/100 g BW), or a standard diet for 1 or 4 weeks (N). After 7 days, liver AMPK phosphorylation is decreased in both HFD and HFD-T2-treated rats with respect to N ones whereas after 30 days the HFD - T2-treated rats show a marked increase in liver AMPK phosphorylation. This transient variation in the HFD -T2-treated rats is correlated with the hepatic lipid content which is high after 7 days of treatment and almost absent after 30 days. In WAT, after 30 days, the opposite is true: HFD-T2- treated rats show a marked decrease in AMPK phosphorylation with respect to HFD controls and to N rats. This variation is associated with an increased release of FA by WAT as deduced by its reduced mass. In conclusion, treatment with T2, by increasing FA utilization, results in differential, transient AMPK phosphorylation between a tissue which depends on FA usage (the liver) and one that causes their release (WAT). Research relating to this abstract was funded by Prin Cofin 2004 Protocol number 2004053441_002 and LR n.5 Protocol number 2005.0166044.

T1:PO.75

PPARGC1/PGC-1 Gly482Ser* PPARα Pro12Ala Interaction on Features of the Metabolic Syndrome

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Variants in both genes have been associated with features of metabolic syndrome (MS). To determine whether the Gly482Ser polymorphism of the PGC-1 gene was associated with features of MS in the Spanish population and its interaction with the Pro12Ala PPARG polymorphism, we genotyped 806 unrelated subjects recruited from a crosssectional population-based epidemiological survey in Central Spain (Segovia, Castille).
BMI, WHR. OGTT, fasting and 2 hrs glucose, serum insulin, leptin, adiponectin levels; lipid profile. Insulin resistance by HOMA-IR. Gly482Ser PPARGC1 and Pro12Ala PPARG genotypes determined by PCR-RFLP. MS (2005 IDF criteria).
In the whole population the Gly482Gly genotype was associated with higher WHR (0.91□1.03 vs. 0.89□0.09, *P*=0.035), and a trend toward higher IR (*P*=0.076) compared with Ser482 allele carriers. Among non-obese the presence of MS was higher in those with the Gly482Gly than in Ser482 allele carriers (21.5% vs. 14.4%, *P*=0.032). Impaired glucose tolerance was higher in Pro12Pro homozygous compared to carriers of the Ala12 allele (16.8% vs 8.1%, *P*=0.027) even after adjustment for sex, age, BMI, WHR and IR (OR: 2.5, CI 95% 1.04–6.25, *P*=0.049).
An additive effect between Gly482Gly PPARGC1 and Pro12Pro PPARG genotypes, and a higher presence of MS was detected in non-obese subjects, suggesting a genegene collaboration.
Gly482Gly PPARGC1 variant *per se* might favour predisposition to abdominal obesity and insulin resistance in our population. A possible additive effect between Gly482Gly PPARGC1 and Pro12Pro PPARG genotypes on the presence of MS in non-obese individuals is suggested.

T1:PO.76

Association between genotypes and specific phenotypes of obesity – A comparison of ten specific obesity phenotypes

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Background: The detrimental effects of obesity depend on the body fat dist ribution, which may signify possible etiological heterogeneity as well. Previous association studies on single nucleotide polymorphisms (SNPs) for *UCP2, UCP3, PPAR g, CART, GRL, MC4R, PTP1B, MKKS, SHP, IRS1, GHRL, MCHR1* and obesity have mainly focused on BMI, but with inconsistent results.
Objective: We systematically examined and compared eleven specific abdominal, peripheral, and overall obesity phenotypes with previously published S NPs in the mentioned panel of obesity candidate -genes.
Methods: Analyses were performed using anthropometric and genotype data from a follow –up examination in 1998 -2000 of a population-based case-cohort study of obese men (*n*=234; BMI•31 kg/m^2) and a control group (*n*=323; BMI<31 kg/m^2). Obesity phenotypes included BMI, fat mass (FM), waist, waist|BMI, waist|FM, intraabdominal adipose tissue, sagittal abdominal diameter, hip, hip|waist, and lower body fat. Associations between SNPs and obesity phenotypes were analyzed separately in co-dominant, dominant and recessive genetic models.
Results: The minor allele for *SHP* G171A was associated with large hip in a dominant model (*P*=0.02). The major allele for *UCP2* –866G>A and *MKKS* Arg51Cys was associated with increase d visceral (*P*=0.01) and overall obesity (*P*=0.05) in a recessive model. The minor allele for *IRS1* G972R and *MCHR1* 100213G>A was associated with little visceral obesity in a dominant model (*P*=0.003 and *P*=0.02-0.05, respectively). The major allele for *UCP2* exon8 was associated with little v isceral (*P*=0.01-0.04) and overall obesity (*P*=0.003-0.04) in a recessive model.
Conclusion: We found distinct associations between gene polymorphisms and specific obesity phenotypes, predicting beneficial or detrimental effects, depending on abdominal, peripheral or overall obesity.

T1:PO.77

Relationship of circulating levels and adipose tissue expression of Adiponectin with insulin-resistance in severe obesity

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Severe obesity is associated with muscle insulin-resistance and reduced circulating adiponectin concentrations. We investigated whether other tissues (liver and fat) also are insulin-resistant and whether such insulin-resistance is associated with reduced plasma adiponectin and adiponectin gene expression in visceral (VAT) and subcutaneous (SAT) adipose depots. Eleven severely obese non-diabetic women (OB, BMI=51±2kg/m^2) and 7 lean women (CT, 23±1kg/m^2) received a euglycaemic-hyperinsulinemic clamp with measurement of endogenous glucose production (EGP, by 6,6-2H2-glucose) and lipolysis (as release of glycerol, RaGLY, by 5H-glycerol). Hepatic resistance (H-IR) was expressed as EGP*x*Fasting Insulin and adipose tissue insulin-resistance (AT-IR) as RaGLY*x*Fasting Insulin. Adiponectin expression in VAT (APNVAT) and SAT (APNSAT) was measured by Real-Time PCR in tissue specimens obtained during bariatric surgery (gastric by-pass) in 8 obese subjects. Compared to CT, OB were more insulin-resistant at the whole-body level (M value:33±5 *vs* 59±8µmol.min^{-1}.kgffm $^{-1}$, *P*=0.01), liver (HIR:294±38 *vs* 111±5µmol.kgffm $^{-1}$.min^{-1}.[mU.L^{-1}], *P*=0.003) and adipose tissue (AT-IR:8.7±1.9 *vs* 0.9±0.2mmol.min^{-1}. [mU.L^{-1}], *P*=0.01). Plasma adiponectin levels were decreased in OB (5±1*vs*8±1ng/ml, *P*=0.02) and were inversely related to H-IR (*r*=-0.59, *P*=0.01), AT-IR (*r*=-0.56, *P*=0.02), RaGLY (*r*=-0.49, *P*=0.04) and directly related to M values (*r*=0.77, *P*=0.0003). In OB, APNVAT expression, but not APNSAT, was directly related to plasma adiponectin (*r*=0.78, *P*=0.03) and M (*r*=0.88, *P*=0.004). In conclusion, in morbidly obese subjects insulin-resistance is present at all levels (muscle, liver and adipose tissue) and is associated with decreased levels of circulating adiponectin. Plasma adiponectin is the principal determinant of peripheral insulin-sensitivity. Reduced adiponectin expression in visceral, but not subcutaneous, fat is associated with peripheral insulin-resistance.

T1:PO.78

Expression of P2 purinergic receptors in human adipose tissue

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BACKGROUND: Extracellular nucleotides have been identified as important signaling molecules regulating physiological functions almost in all cells acting on specific plasma membrane receptors named P2 purinergic (P2R). These receptors can f orm ion-channels or activate a signal transduction pathway via G -protein involvement. Obesity is a chronic low grade inflammatory condition associated with an abnormal cytokine production and increased macrophage infiltration. Although a role of P2R in inflammation has been highlighted, the expression pattern of these receptors in different adipose tissue depots and in adipocyte cultures has not been studied. The aim of the present study was to investigate the role of the purinergic system in adipose tissue.
METHODS: RT -PCR was utilized to detect the expression of the different P2R subtypes in visceral and subcutaneous adipose tissue from healthy and obese subjects. P2R expression profile was analyzed during *in vitro* adipogenic differentiation. Cytokine secretion was detected with immuno-enzymatic assay. ATP released from cell cultures was measured by luminescent assay.
RESULTS: We identified almost all subtypes of P2R in human adipose tissue. Interestingly only two were differently expressed in preadipocytes and in mature adipocytes, both *in vitro* and *ex vivo*. Human adipocytes secreted IL -6 after stimulation with extracellular ATP in a dose dependent manner. Furthermore we revealed that human adipocytes were able to tonically release ATP.
DISCUSSION: The results of the present study show that the purinergic system is present in fat cells and could be involved in the mechanisms modulating the inflammatory response in obesity.

T1:PO.79

Postnatal development of Zinc-alpha2-glycoprotein expression in rat adipose tissue

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BACKGROUND: Extracellular nucleotides have been identified as important signaling molecules regulating physiological functions almost in all cells acting on specific plasma membrane receptors named P2 purinergic (P2R). These receptors can form ion-channels or activate a signal transduction pathway via G -protein involvement. Obesity is a chronic low grade inflammatory condition associated with an abnormal cytokine production and increased macrophage infiltration. Although a role of P2R in inflammation has been highlighted, the expression pattern of these receptors in different adipose tissue depots and in adipocyte cultures has not been studied. The aim of the present study was to investigate the role of the purinergic system in adipose tissue.
METHODS: RT -PCR was utilized to detect the expression of the different P2R subtypes in visceral and subcutaneous adipose tissue from healthy and obese subjects. P2R expression profile was analyzed during *in vitro* adipogenic differentiation**.** Cytokine secretion was detected with immuno-enzymatic assay. ATP released from cell cultures was measured by luminescent assay.
RESULTS: We identified almost all subtypes of P2R in human adipose tissue. Interestingly only two were differently expressed in preadipocytes and in mature adipocytes, both *in vitro* and *ex vivo*. Human adipocytes secreted IL -6 after stimulation with extracellular ATP in a dose dependent manner. Furthermore we revealed that human adipocytes were able to tonically release ATP.
DISCUSSION: The results of the present study show that the purinergic system is present in fat cells and could be involved in the mechanisms modulating the inflammatory response in obesity.

T1:PO.80

Mechanism of leptin expression in breast cancer cells: Role of hypoxia-inducible factor-1alpha

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We reported previously that the obesity hormone leptin is overexpressed in breast cancer biopsies. Here we investigated molecular mechanisms involved in this process. We found that the leptin promoter is activated by high doses of insulin and/or hypoxia, conditions that are associated with obesity. These treatments also increased nuclear accumulation of hypoxia-inducible factor 1α (HIF-1α). In breast cancer cells, HIF-1α associated with upstream leptin gene regulatory sequences, especially with the proximal promoter containing four hypoxia response elements and three GC-rich regions. Loading of HIF-1α to the proximal leptin promoter coincided with corecruitment of p300, the major HIF coactivator. Association of HIF-1α/p300 with the leptin promoter was followed by increased leptin mRNA and protein expression. Conversely, downregulation of HIF-1α with RNA interference totally abolished leptin expression. The highest levels of nuclear HIF-1α, the greatest association of HIF-1α/p300 with the proximal leptin promoter, and the maximal leptin mRNA and protein expression were induced by combined insulin and hypoxia treatments. Our results suggest that targeting HIF-1α might be essential in the treatment of leptin-overexpressing breast tumors.
Research relating to this abstract was funded by the WW Smith Charitable Trust and Sbarro Health Research Organization.

T1:PO.81

Regional differences in the response of adipose tissue to short-term food deprivation in the rat

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The adipose tissue undergoes important adaptations during fasting; however differences in metabolic activity of adipocytes exist depending on the localization of the tissue. Thus, we have studied the effect of short-term food deprivation on the expression of genes involved in lipid metabolism in adipose tissue from different anatomical localization in the rat. We studied adult male Wistar rats under feeding conditions, and after 4-, 8- or 24-h fasting, and analysed by RT-qPCR the expression of genes involved in lipogenesis (FAS, ACC1, GPAT), lipolysis (CPT-1), PPAR-gamma, and GLUT-4 in subcutaneous (inguinal) and in visceral (retroperitoneal and mesenteric) white adipose tissue depots. Fasting induced a decrease in the size of fat depots that was higher for the retroperitoneal depot, followed by the mesenteric and finally by the inguinal depot. In the different fat pads, fasting was accompanied by a decreased rate of fat synthesis, as evidenced by a decrease (after 8-h fasting) in the expression of lipogenic enzymes. However, mRNA levels of CPT-1 increased after 24-h fasting in the retroperitoneal and mesenteric fat depots, while decreased in the inguinal depot. This was parallel to a decrease in PPARgamma expression levels (already after 4-h fasting) in the retroperitoneal depot and of GLUT-4 (after 8-h fasting) in the retroperitoneal and mesenteric depots, while the expression of these genes was not significantly changed in the inguinal depot. In summary, these results agree with different functionally and metabolic activity between subcutaneous and visceral adipose tissues (the latter more oxidative) that are further enhanced during starvation.

T1:PO.82

Effect of acute treatment with retinoic acid on lipid metabolism in liver of mice

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INTRODUCTION: Vitamin A, in retinoic acid (RA) form, modulates the de velopment and function of adipose tissues. A poor status in vitamin A favors an increase of adiposity and adipogenic/lipogenic capacity in adipose tissues. On the other hand, acute treatment with RA and dietary vitamin A supplementation favour a reduction of adiposity and an increase of the thermogenic capacity in adipose tissues and muscle. The liver plays a pivotal role in lipid metabolism in the organism, and it is involved indirectly in growth and activity of adipose tissues.
OBJECTIVE: To investigate the effect of acute treatment with RA on hepatic lipid metabolism.
METHODS: NMRI mice were fed with standard diet and treated with all *-trans-*RA (10, 50, 100 mg/kg animal) or vehicle (olive oil) for 4 days before sacrifice. Body weight, adiposity, food intake, liver composition, circulating biochemical parameters and expression of key mRNA species in liver (by semiquantitative RT - PCR) were determined.
RESULTS: We observed an induction of mRNA levels of genes involved in oxidative metabolism, and a decrease of genes that control lipid biosynthesis. These changes were correlated with a decrease in circulating triglycerides (mainly from VLDL) and an increase of ketone bodies.
CONCLUSION: These results suggest that RA is able to stimulate lipid oxidation and inhibit lipid biosynthesis in liver. These effects could contribute to the adiposity reduction observed after administration of RA.

T1:PO.83

The level of advanced oxidation protein products (AOPP) increases along with the deepening of metabolic disorders in obese children

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Metabolic syndrome (MetS), found already in childhood, is involved in the de velopment of atherosclerotic cardiovascular disease and type 2 diabetes mellitus . Although cardiovascular disease in adults has been linked with oxidative stress, data on i ts involvement in children's obesity is scarce. We evaluated AOPP, a new oxidative status marker, and malonodialdehyde concentrations in 21 controls, 6 overweight and 129 obese children. Patients were divided into groups without and with MetS (MetS - and MetS+) according to IDF criteria. Serum AOPP level was determined according to Witko-Sarsat method. Differences in AOPP and malonodialdehyde levels between groups were analyzed with Mann-Whitney *U* test, while correlations with HOMA -IR, FGIR, fasting glucose (FG) and insuline (F I), Cpeptyde, BMI, waist and hips circumferences, HbA1c with Spearman test. The lowest AOPP and malonodialdehyde levels were observed in controls (63 mmol/l and 1.2 nmol/ml, respectively), insignificantly elevated in overweight subjec ts (67.5 mmol/l and 1.3 nmol/ml) and significantly higher in obese individuals (115 mmol/l, $P= P<0.001$ and 1.5 nmol/ml, $P<0.001$). Among obese subjects, AOPP levels tended to be elevated in MetS+ patients as compared to MetS - (126 *vs.* 95 mmol/l, $P=0.295$), while malonodialdehyde levels were not (1.43 *vs.* 1.45 nmol/ml, $P=0.341$). AOPP level correlated with HOMA ($R=0.224$, $P=0.014$), FGIR ($R= -0.206$, $P=0.024$), FI ($R=0.210$, $P=0.022$), waist circumference ($R=0.244$, $p=0.021$), correlation with C -peptyde was nearly significant ($R=0.174$, $P=0.083$), while no correlation with FG, BM I, hips circumference and HbA1c was observed. Our findings may imply that impairment of oxidative status observed in childrens' obesity increases along with the deepening of metabolic disorders.

T1:PO.84

Human adipose 11beta-HSD type 1 gene expression is downregulated by PPAR-gamma agonists

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Background: 11β-HSD1 is considered a major determent for local activity of glucocorticoids in adipose tissue, and has been associated with the metabolic syndrome. Peroxisome proliferator-activated-receptor-γ (PPAR-γ) has been shown to play a role in adipocyte metabolism and PPAR-γ agonists have been shown to downregulate 11β-HSD1 in 3T3-L1 adipocytes in vitro and in mice in vivo. Little is known about the effects of PPAR-γ agonists on 11β-HSD1 expression in human adipose tissue, therefore we investigated the effects of PPAR-γ on 11γ-HSD1 gene expression in human adipose tissue both *in vivo* as well as *in vitro*.
Methods: For the in-vitro study, adipose tissue from 10 healthy women sampled during liposuction was incubated with troglitazone(10nM) in the presence of IL-1γ, a known potent stimulator of 11β-HSD1 for 48 hours. For the in vivo study, 6 obese men were treated for 3 months with rosiglitazone (4mg/day). Adipose tissue biopsies were obtained before and after treatment. There were no significant changes in weight, total body fat, or measures of insulin resistance.
Results: Treatment with rosiglitazone for 3 months resulted in a reduction in 11β-HSD1 expression of approximately 60% in obese men ($P>0.001$). In the in vitro study, incubation with IL-1 amplified human adipose tissue 11β-HSD1 by approximately 150%($P<0.001$), but addition of troglitazone reduced the IL-1γ induced 11β-HSD1 expression by 41%($P>0.05$) compared to controls.
Conclusion: Human adipose 11β-HSD1 expression is downregulated by PPAR-γ agonists in vitro and in vivo and accordingly PPAR-γ activation may be able to reduce the amount of locally produced cortisol in adipose tissue.
1. Conflict of Interest: None Disclosed.
2. Funding: Research relating to this abstract was supported by the Danish Medical Research Council, The Clinical Institute, Aarhus University and the Novo Nordic Foundation.

T1:PO.85

PAI-1 adipose tissue gene expression in obesity may be linked with thyroid hormones

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The metabolic syndrome is associated with an increased risk of developing cardiovascular disease, and PAI-1 overexpression may participate in this process. The positive correlation b etween PAI-1 adipose tissue gene expression and serum concentration has been observed. Increased PAI -1 blood levels enhance the risk of atherothrombosis. It has been shown in obese children high serum levels of thyroid hormones (T3 and T4) and PAI -1. The aim of this study was to analyse the effect of T3 and T4 treatment on PAI -1 gene expression by 3T3-L1 adipocytes. Cells were treated with T3 or T4 (10 or 100nM) for 24 o r 48 hours. Cells were harvested and RNA extracted with Trizol. PAI-1 mRNA quantificatio n was performed with Real Time PCR. The 24 hours treatment with 100nM T3 increased the PAI -1 mRNA as compared to the control (241,61 ± 30,2 vs. 100,51 ± 10,9). The same was observed on the 48 hours treatment with 10nM T4 (289,5 ± 46,0 vs. 100,0 ± 5,5). The se results show that thyroid hormones stimulate PAI-1 adipose tissue gene expression in cultured cells, and it may be suggested that there is a pos itive correlation between PAI-1 blood levels and thyroid hormone s in human beings. Supported by FAPESP and CN Pq.

T1:PO.86

Effects of maternal diet on the expression of fatty acid transporter proteins FATP1 and FATP4 in the offspring

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The nutritional status of the mother during gestation and early lactation may affect fatty acid metabolism in the o offspring. Fatty acid transport proteins (FATP) play an important role in the cellular uptake of fatty acids. In this research we studied the impact of maternal diet on mRNA expression levels of FATP4 in the intestine and FATP1 in adipose tissue. Three groups of female Wistar rats (n=28 -32) were kept on a control diet (C), a high fat/high calorie (H) or a restricted/ low calorie (L) diet during 6 weeks premating, mating, gestation and lactation. Immediately after birth (postnatal day (PN) 1), F1 -pups were cross-fostered to dams of the same group or to dams of the other two dietary groups resulting in 9 different groups and fed the various diets up to PN 70. At PN01 and PN70 animals were sacrificed and FATP4 and FATP1 mRNA expression levels determined. Food consumption, energy intake and body weight of the dams differed significantly between the groups . The results showed that 1) at birth, pups of dams on a high and low calorie diet showed higher FATP4 and lower FATP1 levels as compared to the controls 2) apparently, FATP4 expression levels in the intestine decreased from birth onwards to PN 70 3) Differences in FATP4 and FATP1 expression levels were larger in male than in female offspring 4) Differences in maternal diet affected both FATP4 and FATP1 expression levels in intestine and adipose tissue, respectively. In conclusion, maternal diet manipulation affects FATP1 and FATP4 expression levels in the offspring.

T1:PO.87

Arachidate induces hypothalamic inflammation through mechanisms dependent on TLR2 and TLR4 activation and endoplasmic reticulum stress

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Consumption of fat -rich diets induces the hypothalamic resistance to insulin and leptin (Endocrinology 146: 4192, 2006). Some of this effect is due to the activation of proinflammatory signaling in the hypothalamus. The objective of this study was to evaluate the role of distinct fatty -acids in the induction of inflammation in the hypothalamus. Initially, Wistar rats were icv cannulated and treated with albumin, oleic acid, saturated fat mixture (stearic, palmitic, arachidate and behenic acids - 25:25:25:25), or vegetable oil mixture (oleic, linolenic, linoleic acids and the mixture of saturated – 20:30:30:20). Highest expressions of the pro -inflammatory cytokines TNF - , IL-1, IL-6 were observed after five days treatment with the saturated fat mixture. To further explore the role individual saturated fatty acids in the induction of hypothalamic inflammation, rats were icv treated with each of the saturated fatty acids. Highest expression of inflammatory cytokines was detected with arachidate. To explore the mechanisms involved in arachidate -dependent induction of inflammatory response in hypothalamus, icv cannulated rats were treated with arachidate and samples of hypothalami were obtained after 0 –5 days for evaluation of induction of endoplasmic reticulum stress (GRP78, phospho -elf2a, phospho -PERK and phospho -JNK) and activation of T LR2 and TLR4 signaling (association of TLRs with Myd88). Both, the induction of endoplasmic reticulum stress and the activation of TLR2 and TLR4 signaling were detected after 3 days of icv arachidate treatment. Thus, arachidate appears as an important hypo thalamic pro-inflammatory factor that may play a role in diet -induced resistance to anorexigenic signals.

T1:PO.88

Characterization of the transcriptional control of the human CIDEA gene

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Background: Obesity and peripheral insulin resistance are risk f actors for development of non-insulin dependent diabetes mellitus (NIDDM) but the molecular mechanisms underlying the association remains to be defined. We recently described a gene, cell death-inducing DFFA (DNA fragmentation factor -a)-like effector A (CIDEA) with important effects on human adipocytes and with a possible protective role in insulin resistance. CIDEA mRNA expression in adipose tissue is reduced in obesity and correlates negatively with features of the metabolic syndrome. Both *in vitro* and *in vivo* data suggest that downregulation of CIDEA may be important for the development o f insulin resistance. The transcriptional regulation of *CIDEA* in human cells is unknown.
Aims: To investigate the transcriptional regulation of human *CIDEA*.
Methods: A 1340bp genomic fragment upstream of the transcriptional start site was cloned. Fragments of different size were generated and inserted into the luciferase reporter vector pGL3-basic. Transcriptional activity of promoter constructs was analysed in 3T3 -L1 cells.
Results: Bioinformatic analysis of the 1.34kb sequence revealed a number of putative transcription factor binding sites. There was an inverse relationship between luciferase activity and the length of the fragments suggesting that inhibitory regions may be present in the region -1340-500bp. Detailed analysis of multiple single nucleotide polymorphisms and corresponding haplotypes in the promoter are now under way.
Conclusion: We were able to define the minimal promoter required for *CIDEA* transcription in a 1.34kb long sequence upstream of transcription start site of the *CIDEA* gene. These results are important for future modulatory effects on C IDEA expression.

T1:PO.89

Association of TNF-a -308 G>A polymorphism with obesity and lifestyle parameters in children 10-12 years

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Several studies have suggested an association between Tumor necrosis factor - alpha -308 G>A polymorphism (TNF -a) and obesity related phenotypes. Moreover, there are environmental factors, such as eating frequency (EF) and physical activity that have also been found to influence obesity status. Aim of the present study was to evaluate possible interactions between TNF -a -308 G>A polymorphism, obesity and lifestyle parameters in a randomly selected group of children 10 –12 yrs, who enrolled in the Gene -Diet Attica Investigation on childhood obesity (GENDAI). These children underwent full nutritional assessment and biochemical measurements. For the present study, the above –mentioned associations were evaluated in a subsample of 472 children. Genotyping was performed by PCR- RFLP analysis. The genotype frequencies were 85% (n=401), 14.8% (n=70) and 0.2% (n=1) for G/G, G/A, and A/A respectively. T he distribution of TNF-a -308 G>A polymorphism did not differ between normal weight, overweight and obese children. Body Mass Index (BMI) was positively correlated with duration of inactivity (minutes) in GA subjects (Spearman rho =0.302, P = 0.012), while no correlation was found for GG subjects. BMI was negatively correlated with EF only in GG subjects (rho= -0.202 in GG subjects, p<0.001 vs. rho = 0.013, P=0.9 in GA subjects). Univariate analysis revealed an interaction between TNF -a -308 G>A polymorphism and EF as well as between TNF-a -308 G>A polymorphism and inactivity, after controlling for potential confounders. In conclusion, these preliminary results indicate that TNF -a-308 G>A polymorphism may be associated with lifestyle parameters and could, thus, influence obesity related phenotypes.

T1:PO.90

Genetic markers as cardiovascular risk factors in obese children

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Many studies performed in adults have reported the implication of genetic determinants in the occurrence and the progression of arterial alterations. To date, no study evaluated this association in children. The aim of the study was to search for genetic markers susceptible to favor the occurrence of early arterial alterations in obese children. Subjects and methods: 232 obese children (150 girls; 11.4 ± 3.1 years; BMI z-score 4.6 ± 1.2 SD) have been studied. We performed an association study between arterial parameters and polymorphisms in genes coding for protein involved in the renin-angiotensin system (angiotensin I-converting enzyme (ACE I/D), angiotensinogen (AGT G-6A and Met235Thr) and angiotensin II type1 receptor (AGTR1 A1166C)); vascular endothelial cell remodelling (Stromelysin 1 (MMP3 5T/6T), plasminogen-activator inhibitor type1 (PAI-1 4G-668/5G), communication (connexin CX37 Pro319Ser) and inflammation through leukocyte adhesion (fractalkine receptor (CX3CR1 Val249Ile); arterial vasodilation (NOS3 Glu298Asp); adiponectin plasma levels (APM1 C-11377G and A- 11391G) and lipoprotein metabolism (Paraoxonase 1 (PON1 Glu192Arg). Nonivasive arterial measurements were performed to evaluate the intima-media thickness, the common carotid artery compliance (CCA) and distensibility (CCD), the incremental elastic modulus (Einc), and the endothelial function. Results: among all the polymorphisms studied, only a trends of association between the C allele (additive model) of the angiotensin II type1 receptor and CCA (P=0.13), CCD (P=0.09) and Einc (P=0.07) was observed. Conclusion: these results suggest the AGTR1 A1166C may be involved in obese children early arterial alterations. The replication of this study in a larger cohort is necessary to confirm our result.

T1:PO.91

Association between Glutamate Decarboxylase 2 (GAD2) and binge eating in obese children: results from the GENDAI study

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There is limited evidence on the role of gene tic and environmental factors on the etiology of childhood obesity, a major health problem worldwide. Recently, polymorphisms in the Glutamic Acid Decarboxylase 2 Gene (GAD2) and specifically the functional -243A>G polymorphism in the 5′ promoter region has been associated with adult morbid obesity and childhood obesity. The Gene -Diet Attica Investigation on childhood obesity (GENDAI) is a school -based cross-sectional study. It evaluates the contributions and pivotal interactions of genetic, dietary and physical activity variables on children's overweight. Anthropometric, clinical genetic, socio -demographic, other lifestyle characteristics were collected from participating children. A full dietary assessment w as obtained including a questionnaire on meal patterns and behaviors related to the act of eating, including binge eating behavior. Potential associations between GAD2 polymorphism and binge eating were evaluated in a subsample of 920 children. No association was found between GAD2 (-243A>G) and obesity status (normal weight, overweight and obesity) in children. With regards to binge eating, no association was found between GAD2 and binge eating in normal weight children, while in overweight/obese children a trend towards binge eating (P=0.076) was found in the G allele carriers with 42.9% of GG obese/overweight carriers vs. 21.8% of AA obese/overweight carriers reporting binge eating phenotype. When the two sexes were examined separately, this trend was found only in overweight/obese boys (P=0.073) but not in overweight/obese girls (P=0.673). These preliminary data indicate that GAD2 may play a role in the complex mechanisms underlying energy balance and food intake in overweight children. No conflict of interest Research relating to this abstract was funded by COCA-COLA HELLAS

T1:PO.92

SNPs in adipokines - adiponectin and leptin - as risk factors for diabetic complications: evidence for a "double-hit" mechanism?

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Diabetic angiopathy is now recognized as an inflammatory process of the vessel wall. Type 2 diabetes (T2DM) is far the most common type frequently accompanied by obesity in affected subjects. Co -occurrence of both diseases is not just an epidemiological association but a likely consequence of common ethiopathogenesis. Adipokines too play multiple roles in inflammation ultimately activating, similarly to hyperglycemia itself, transcription factor NF kB with subsequent inflammatory changes in vascular tissue. Leptin (*LEP*) and adiponectin (*APM1*) are, apart of their role in energy homeostasis, examples of vascular pro - and anti-inflammatory mediators, respectively. Genetic variability in the *APM1* and *LEP* genes could not only modulate the risk of obesity and/or T2DM but also influence susceptibility to the development of diabetic complications. The aim was (i) to analyse association between common functional SNPs *APM1* 94T/G and *LEP* -2548G/A with diabetic nephropathy in the pilot study and (ii) using additional more densely spaced SNPs in both genes to ascertain event. haplotype association (ongoing). The cross-sectional study comprised 331 T2DM patients with normoalbuminuria (n=146, age 59.5±14.6 yrs) or diabetic nephropathy (n=185, age 65 .2±14.6 yrs). Genotypes of *APM1* 94T/G and *LEP* -2548G/A were detected by PCR -based methodology. Allele frequencies of the two SNPs did not differ significantly (P>0.05, chi-square test), nevertheless, both SNPs exhibited statistically significant deviations from Hardy - Weinberg equilibrium in the nephropathy group (P<0.05, chi -square test). Preliminary results indicate that carriers of certain variants in *APM1* and *LEP* genes might be susceptible to diabetic nephropathy. Results of ongoing haplotype analysis will be thus very informative. Conflict of Interest: None Disclosed.
Funding: Research relating to this abstract was funded by grant KJB501620601 from the Grant Agency of the Czech Academy of Sciences.

T1:PO.93

Adipose tissue stearoyl-CoA desaturase 1 (SCD1) gene expression in obese subjects

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SCD1 is a central lipogenic enzyme which catalyses the desaturation of long-chain fatty acids and its predominant sites of expression are adipose tissue (AT) and liver. In liver SCD1 has been shown to be repressed by leptin. The role of SCD1 in modulating lipid and glucose metabolism in AT is not clear. The aim was to examine SCD1 gene expression in AT in obese subjects. Seventy-five (37 males, 38 females) obese subjects (60±7 yrs, BMI 32.9±2.8 kg/m^2, mean±SD) with IGT and features of metabolic syndrome, were recruited. Fasting blood samples were drawn to determine concentrations of plasma glucose, insulin, total lipids and inflammatory markers. SCD1 mRNA expression was studied in abdominal subcutaneous AT using real-time PCR. Gender difference in SCD1 mRNA expression was evident (P=0.004) with 84±31 AU in women and 54±50 AU in men. In the whole study population a positive correlation between SCD1 mRNA levels and percentage of body fat (r=0.447, P<0.001) and body fat mass (r=0.320, P=0.007) was found. In addition, positive correlations, independent of BMI and gender, were found with sterol regulatory element binding protein (SREBP) -1c (r=0.376, P=0.002) and leptin mRNA levels (r=0.345, P=0.004) and with fS-leptin (r=0.260, P=0.034). In women, serum CRP and SCD1 gene expression correlated r=0.457 (P=0.006). This is the first study to demonstrate a clear gender difference in SCD1 ene expression women showing higher mRNA levels. The found associations suggest a role for SCD1 in the development or consequences of obesity, but also in the regulation of adipose tissue metabolism.

T1:PO.94

A proximal cAMP response element differentially regulates PGC-1α gene expression in brown and white adipose tissue cell lines

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Background: PKA dependent induction of PGC -1alpha and UCP1 expression is an essential step in the commitment of pread ipocytes to the brown adipose tissue (BAT) lineage. In HIB1B (BAT) cells PGC -1alpha and UCP-1 expression is cAMP-inducible, but in 3T3 -L1(WAT) cells, expression is cAMP -insensitive. Our aim was to characterize the proximal CRE in the PGC -1alpha promoter and identify whether the cell -specific differential expression is due to different combinations of transcriptional factors binding to the CRE in response to the cAMP stimulation in BAT and WAT cell lines.
Methods: Nuclear extracts were prepared from conflue nt HIB-1B and 3T3-L1 cells treated with forskolin to stimulate cAMP. Nuclear protein binding to the CRE region in the proximal PGC -1alpha promoter was then determined using electro-mobility shift assays and chrom atin immunoprecipitation experiments .
Results: Gel shift experiments demonstrated that CREB, ATF -2, C/EBPbeta but not C/EBPalpha, bound to the CRE region in nuclear extracts from HIB -1B cells, but only CREB from 3T3 -L1 nuclear extracts was able to bind on the same CRE. Chromatin immunoprecipitation studies demonstrated that C/EBPbeta and phosphoCREB bound to the CRE region in HIB-1B cells but binding of these transcriptional factors was much less in 3T3 -L1 cells.
Conclusions: Our results demonstrate that different combinations of transcription factors binding to the proximal CRE of PGC-1alpha may be responsible for the differential response of PGC-1alpha expression to cAMP in brown versus white adipose tissue.
1. Conflict of Interest: None Disclosed.
2. Funding: Research relating to this abstract was funded by the BBSRC and Greek scholarship foundation (IKY) for funding.

T1:PO.95

Association of sequence variations in the gene encoding adiponectin (ADIPOQ) with body size, metabolic parameters and serum adiponectin levels. The Finnish Diabetes Prevention Study

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Background and aims: Adiponectin, encoded by the *ADIPOQ* gene, is an adipose tissue derived circulating peptide with insulin -sensitising and anti -atherogenic effects. Low serum adiponectin levels are associated with obesity, insulin resistance and type 2 diabetes. Our aim was to study association of the *ADIPOQ* gene variations with body size, glucose metabolism and serum adiponectin levels in subjects with impaired glucose tolerance (IGT).
Subjects and Methods: Altogether 507 subjects participating in the F innish Diabetes Prevention Study (DPS) were genotyped for six single nucleotide polymorphisms (SNPs) in the *ADIPOQ* gene, and associations between SNPs and baseline anthropometric and metabolic parameters were studied. In addition, in a subgroup of subjects (*n*=237), association between SNPs and baseline serum adiponectin levels were analysed.
Results: The SNPs rs1501299, rs382179 9 and rs6773957 were significantly associated with body weight, sagittal diameter, fasting insulin level and HOMA -IR. The SNP rs2241766, was associated with serum total and HDL cholesterol levels. In a subgroup analysis, differences in baseline serum adiponectin levels were seen according to SNPs rs17366568, rs2241766 and rs6773957.
Conclusions: The present findings suggest th at genetic variation in *ADIPOQ* contributes to body size, fasting insulin, total and HDL cholesterol levels and serum adiponectin levels in DPS population.
Research relating to this abstract was funded by the Finnish Academy (211497), Sigrid Juselius Foundation and EVO-fund of the Kuopio University Hospital (5106 and 5198).

T1:PO.96

Fatness and fitness: the impact of fitness on the genetic variation of total and abdominal fat

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Aims: The aims of this study are 1) to elucidate the relative importance of genes and environment on variation in total and abdominal fat, and 2) to assess the effect of adjusting for fitness on the total and the additive genetic variation of these two fat measures.
Materials and Methods: 152 female (79 MZ, 73 DZ) and 103 male (58 MZ, 45 DZ) twinpairs of 18-57 years underwent anthropometric measuring and a fitness test. Total fat in percent (fat%BMI) was assessed by BMI, weight and age. Waist circumference was used as a measure of abdominal fat.
Results: Multivariate analysis methods were applied to these two measures of fat, adjusting for age and fitness. Waist ci rcumference was also adjusted for total fat% BMI to investigate abdominal fatness independently of overall fatness. Adjusting for age, the heritability of fat% BMI was about 70% in both genders. Adjusting for age and overall fatness, the heritability of waist circumference was about 50% in both genders. Adjusting for fitness reduced the total variance of fat%BMI by one third, and most of this reduction was in the additive genetic component. However, the fraction of the total variation due to the genetic variation (the heritability) changed very little. The total variation of waist, adjusted for overall fatness, was not reduced after adjusting for fitness, in both genders.
Conclusions: A large proportion of the high heritability of total body fat (%) is due to genes implicated in fitness. There is little or no association between abdominal fatness and fitness.
Conflict of interest: None disclosed.
Funding: Research relating to this abstract was funded by the Danish Medical Research council, The Danish Diabetes Association, The Danish Heart Association and the Novo Nordisk Foundation.

T1:PO.97

Prostacyclin synthase activity regulates the adipose depot specific IL-6 secretion

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Adipokines interleukin -6 (IL-6) and leptin are often reciprocally regulated, with IL -6 release greater in visceral and leptin from sub -cutaneous white adipose tissue (WAT). The mechanism of such regulation is unclear. We show that cyclo -oxygenase-2 (COX-2) is constitutively expressed in WAT and COX- 2 inhibition, pharmacologically (aspirin: 5 mM or NS -39 : 1 μM) or using tissue from COX-2 deficient mice, reduces IL -6 levels in visce ral tissue. Furthermore, prostacyclin synthase (PGI2S) expression and activity were higher in visceral WAT a nd levels of a prostacyclin metabolite, 6 -keto PGF1-alpha correlated directly with IL -6 and inversely with leptin release. No depot specific difference in p rostaglandin E2 synthase (PGE2S) activity was evident in WAT and neither adipokine showed significan t association with endogenous PGE2 release. Basal adipose IL -6 secretion was independent of changes in intracellular [Ca2+] but sensitive to elevation in intracellular cAMP acting via the prostacyclin (IP) receptor. Mice on a high -fat diet showed reduced l ocal PGI2S activity and IL-6 release and elevated leptin production. However, whole body IL -6 release increased as a result of increased total body fat mass. In conclusion, depot specific differences in IL -6 and leptin release reflect differences in the activity of COX/prostaglandins axis and represent a suitable target for modulation of adipokines.
Research relating to this abstract was funded by the Wellcome Trust, Grant Number 070521/Z/03/Z.

T1:PO.98

Cardiorespiratory fitness and insulin sensitivity in monozygotic twins||discordant and concordant for obesity

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Introduction: Obesity is associated with poor fitness. We determined whether acquired obesity is associated with decreased fitness independent of genetic factors in young adult monozygotic (MZ) twins, discordant and concordant for obesity.
Patients and methods: Twelve obesity-discordant (BMI, weight difference 5.4 kg/m^2, 16 kg) and 9 concordant (1.0 kg/m^2, 2 kg) MZ twin pairs aged 24-27 yr were identified among 658 MZ pairs from the population-based FinnTwin16 study. The subjects were healthy and did not u se any medications. Body composition was determined using DEXA and maximal oxygen uptake (VO2max), working capacity (Wmax) and working efficiency (Wmax/VO2max) using bicycle cardiorespiratory exercise test with gas exchange analysis. Whole body insulin sen sitivity was also measured using the euglycemic hyperinsulinemic clamp technique.
Results: Compared to their lean counterparts, the obese co-twins were less fit, as expressed per kg lean body mass (LBM) and measured by VO2max (50.6 ± 6 .5 ml/kgLBM·min vs. 54.2 ± 6.4 ml/kgLBM·min, for obese vs. lean, P<0.05), and Wmax (3.9 ± 0.5 W/kgLBM vs. 4.4 ± 0.7 W/kgLBM, P<0.01). The obese co-twins also had lower mechanical efficiency (22.0 ± 1.4% vs. 23.2 ± 1.3% respectively, P<0.05) and insulin sensitivity than their lean counterparts (M-value 6.1 ± 2 .0 mg/kgLBM•min vs. 9.2 ± 3.2 mg/kgLBM min, P<0.01).
Conclusion: Acquired obesity decreases VO_2max, Wmax, mechanical efficiency and insulin sensitivity independent of genetic influences in MZ twins.

T1:PO.99

Changes serum leptin levels in women with different grades of obesity

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Background: Plasma levels of leptin, the recently discovered satiety hormone, are associated with adiposity in humans. The aim of this study was to determine whether Leptin concentration varies in different grades of obesity.
Material and Methods: Two hundreds of white healthy and obese women were entered in this study. The mean age was 29.76 ± 12.80 years. BMI was calculated as body weight divided by height squared (kilograms per meter squared). Grading of obesity was considered on BMI bases. Normal was BMI 20–25, overweight BMI 25–30, Grade I BMI 30–35, and Grade II BMI 35–40 respectively. Waist-to-hip ratio (WHR) was calculated as the ratio of waist and hip circumferences. Plasma leptin levels were measured by competitive immunoassay kits.
Result: The mean concentration of Leptin in normal and different grades of obese healthy subjects with normal, overweight, grade I, and grade II were 8.03 ± 4.16 ng/ml, 39.30 ± 10.29 ng/ml, 46.60 ±6.38, and 51.11± 14.28 respectively. There is a dramatic increase in Leptin concentration when the BMI was increased. There was statistically significant difference between all groups in leptin concentration (P <0.001). There was a direct and significant association between leptin and BMI in obese subjects (r= 0.67, P< 0.0001).
Conclusion: It is concluded that the concentration of circulating leptin was dramatically increase with increasing BMI and/or different grades of obesity in women. Further elucidation of the potential interaction between endocrine system and obesity could provide important insights for regulation of body weight and the mechanisms leading to obesity.

T1:PO.100

The effect of atorvastatin in Na/Li countertransport of red blood cells in hypertensive dyslipidaemic patients, (preliminary results)

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Indroduction: Increased Na/Li countretransport (Na/Li CT) activity was observed in essential hypertension. It was also found that hyperlipidaemias cause increased red blood cell Na/Li CT activity.
Aim. Investigate the possible effect of atorvastatin in reducing Na/Li CT activity in patients with essential hypertension and dyslipidaemia.
Subjects and methods: 15 patients (7males, 8 females), with mild hypertension and dyslipidaemia (tChol>240mg/dl, Trg<250mg/dl) mean age 49,53±10,46 y, BMI 28,42±2,99, systolic blood pressure (SBP)145,2±9,219mmHg, diastolic blood pressure (DBP) 90,93±7,713mmHg, Chol 282,73±30,27 mg/dl, Trg 164±59,65 mg/dl, HDL 54,46±10,35 mg/dl, LDL 194, 8±27,17 mg/dl with no other disease, not receiving medication, including antihypertensives. All received atorvastatin for 10 weeks, starting with 10 mg/day, adjusted until achieving LDL < 130mg/dl and nondrug antihypertensive treatment. Na/Li CT activity was measured before and after treatment by Canessa method. Ten healthy persons, without family history of hypertension, of similar age and BMI (7 males, 3 females), were controls
Results: Reduction of LDL was statistically significant (194,8±27,17 vs 95,33±12,13 mg/dl, P<0,001). Na/Li CT decreased statistically significant at the end of 10 weeks (0,264567 vs 0,142952 mmolLi/L red blood cell /h, P<0.001), reaching control levels (0,116437 mmolLi/L red blood cell /h). SBP and DBP were statistically significant decreased (145,26 vs 133,2 and 90,93 vs 80 mmHg, P<0,001 and P<0,001 respectively). Na/Li CT reduction was independent of final cholesterol (r: -0.435, P=0.1)
Conclusions: Atorvastatin beyond its hypilipidaemic effect, decreased significantly Na/Li CT activity and both SBP, DBP. Completion of our study might confirm this effect.

T1:PO.101

Partitioning of fuel toward utilization in brown adipose tissue in cancer cachexia

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Profound loss of adiposity in cancer cachexia is thought to be induced by decreased food intake and/or increased energy expenditure and catabolism. Brown adipose tissue (BAT) activation through mitochondrial biogenesis and oxidative metabolism contributes to energy expenditure. Although BAT activity is implicated to be increased in cancer cachexia, the molecular mechanisms remain unclear. We examined BAT morphology and the transcription of specific genes involved in mitochondrial function of BAT in a mouse model of cancer cachexia. MAC16 tumour was transplanted into the flank of NMRI mice to induce cachexia. A pair -fed group was introduced to exclude the e ffects of reduced feeding. Using light and electron microscopy, BAT from cachectic mice appe ared to be more vasculature with reduced lipid content in brown adipocytes when compared with non tumour-bearing controls. Quantitated by real -time PCR, mRNA levels of fat storage enzymes including fatty acid synthase, acetyl CoA carboxylase and stearoyl CoA desaturase-1 fell markedly. In contrast, there were increases in expression levels of nuclear encoded genes (peroxisome pro liferators-activated receptor gamma coactivator -1 alpha and nuclear respiratory factor -1), uncoupling proteins (UCP -1, -2 and -3) and cytochrome coxidase-4, involved in mitochondrial biogenesis and respiration, as well as the rate-limiting enzyme in fat oxidation (carnitine palmitoyl transferase-1) in BAT of cachectic mice. These changes were independent of mild reduction in food intake in tumour-bearing animals. Our results suggest that the tumour burden favours lipid utilization in BAT probably through enhanced mitochondrial biogenesis and fatty acid oxidation, which prevents triglyceride storage in cancer cachexia.

T1:PO.102

Insulin pathway is blunted in liver from rats maintained in soybean diet

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Maternal malnutrition was shown to lead permanent alterations in the glucose homeostasis of offspring at adult life and the consumption of soy-based foods is associated to beneficial effects on glucose tolerance. We investigated the effect of nutritional recovery with a soybean flour diet on the glucose tolerance, liver glycogen concentration and the expression of some proteins related to insulin action in the hepatic tissue. Rats from mothers fed with 17% or 6% protein (casein) during pregnancy and lactation were maintained with 17% casein (CC and CR groups) or soybean (SC and SR groups) diet after weaning until 120d age. Area under the glucose curves (mg/dl.120min) during the i.p. glucose tolerance test was not significantly different among the four groups (CC:19331±2034, SC:18303±353, CR: 18311±1279, SR:18816±1567). In the fed state, liver glycogen level (mg/100mg tissue) was lower in CR than in CC group (5.94±0.87 and 2.03±0.65, respectively) and the soybean diet significantly reduced the liver glycogen content in both groups (SC: 0.45±0.32, SR: 0.47±0.31).The insulin receptor and insulin receptor substrate-1 levels were lower in recovered than in control rats and in those fed with soybean diet. The Akt protein expression did not differ among groups. These results suggest that the reduction of liver glycogen levels in the fed state is due, at least in part, to alterations in the early steps of the insulin signal transduction pathway. Brazilian Foundations: FAPEMAT e CNPq.

T1:PO.103

Soybean diet reduces fat liver content and ATP citrate lyase but not alter the PPARg protein expression

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Epidemiological and experimental data indicate that malnutrition in the fetal and neonatal life may programme offspring susceptibility to later development of obesity and type 2 diabetes. The rising prevalence these diseases in the population have been considered responsible for the high number of individuals with hepatic steatosis. The consumption of soy protein regulates the expression of molecular mediators of hepatic steatosis preventing the development of fatty liver. We investigated the effect of nutritional recovery with a soybean flour diet on liver fat concentration, the malic enzyme and ATP citrate lyase activities and the expression of PPARg in the hepatic tissue. Rats from mothers fed with 17% or 6% protein (casein) during pregnancy and lactation were maintained with 17% casein (CC and CR groups) or soybean (SC and SR groups) diet after weaning until 120d age. The liver fat content (mg/total tissue) was lower in recovered than in control rats and in those fed with soybean diet (CC: 744±436, SC: 357±102, CR: 495± 181 and SR: 250±46).The ATP-citrate lyase (nmol NADP/g protein/min) was decreased in both groups fed with soybean diet in relation to those maintained with casein (CC: 38±12, SC:30±6, CR: 44±24 and SR:24±15). The malic enzyme activity, the serum aminotransferases levels and the PPARg expression did not differ among groups. These data suggest that soybean diet reduces the liver lipogenesis without changing the PPARg protein expression. Financial Support: FAPEMAT e CNPq.

T1:PO.104

Effect of diet supplemented with 5% carbohydrate on haptoglobin gene expression and circulating level in mice

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The circulating levels of several proteins associated with inflammation are raised in diabetes and obesity. The reduction of the fat in the die t is accompanied by increase of carbohydrate content like sucrose and fructose. These data have taken the researc hers to look back to the theory that sugars can be an important factor to the increase of obesity incidence. The aim of this study was to verify the effect of diet supplemented with 5% carbohydrate on the e pididymal and retroperitoneal adipose tissue hapto globin gene expression and circulating level in mouse. One set of mice (30 days-old) received a different type of carbohydrate (glucose, fructose or sucrose) supplemented diet for 8 weeks – chronic group and another set of animals (90 days -old) received th e same diet for 2 days – acute group. Epididymal and retroperitoneal adipose tissues were removed and gene expression was determined by Northern blotting using a chemilumenescent detection. Haptoglobin circulating level was determined by ELISA. The treatment for 2 days increased the retroperitoneal and epididymal gene expression in the glucose group. This parameter was elevated in the fructose group only in the retroperitoneal. The chronic treatment with fructose promoted an increase in the gene expression in the epididymal when compared to the control group. Only the chronic treatment promoted an increase in the haptoglobin serum level. These results indicate that treatment with a small supplementation of 5% with carbohydrates promoted important alterations on the studied parameters and these effects are depends on the type of carbohydrate and time of treatment.

T1:PO.105

Angiogenesis in the mouse models of metabolic syndrome

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Introduction: Obesity, insulin intolerance, hypertension, dyslipidemia and hyperleptinemia, hipoadiponectinemia are the main clinical symptoms of metabolic syndrome leading to lipid disorders, atherosclerosis, diabetes associated with pathological angiogenesis.
Aim of the study: Was to define the possible link between metabolic syndrome biochemical parameters and angiogenesis.
Methods: Three kinds of mice (NZO, NZO/SJL and lhepatocyte RXRα-/-) predisposed to develop parameters of human metabolic syndrome was used. Mice were fed with standard and high fat diet for seven weeks. Weight as well as biochemical serum parameters (glucose, triglycerides, cholesterol, insulin, leptin and adiponectin) were monitored once a week. For angiogenic properties animals were injected subcutaneously with 500•l of matrigel containing (25nM) bFGF at the beginning of the last week of feeding. After six days matrigel plugs were analyzed by immunostaining with the anti-CD31 (PECAM) antibody. The amount of PECAM positive cells, and number of capillaries was counted.
Results: High fat diet increased the mice body weight, serum concentration of glucose, cholesterol, insulin and leptin. Some tendency to decrease adiponectin concentration in serum was observed. Analysis of the angiogenesis parameters in matrigel sections revealed tendency for high fat diet to rise number of vessels with and without lumen as well as infiltrated CD31 (PECAM) positive cells. Angiogenic response was observed especially in the NZO mice.
Conclusions: Feeding with high fat diet resulted in elevation of parameters characteristic for human metabolic syndrome**,** increase of the leptin to adiponectin ratio. The value of such ratio may be predictive for the pathological angiogenesis accompanying insulin resistance. Acknowledgements: Project supported by Polish Committee of Science Grant No: PBZ-MIN- 005/P04/2002/5.

T1:PO.106

Exercise training and diet effects on lipid metabolism in the liver

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***Aim*:** To study the effect of exercise training on plasma fatty acid composition (C18:1/C18:0) to estimate SCD -1 activity in the liver in response to a high carbohydrate diet (CHO).
***Methods*:** Two groups of rats were either treadmill -trained (TR) for 8 weeks or kept sedentary (Sed). Animals were starved for 24 h, refed a stand ard diet (SD) for 24 h, starved for another 24 h and then refed either a SD or a high CHO diet 24 h prior to sacrifice. Body weight (BW), intra -abdominal fat pad weights, plasma ratio of 18:1/18:0 (desaturation index), liver triglyceride content (TAG), and plasma free f atty acid levels (FFA) were measured in all rats.
***Results*:** The high CHO diet resulted in higher ($P < 0.05$) liver TAG, plasma FFA, and desaturation index compare to the SD diet suggesting an increased hepatic lipogenesis activity. Fat pad weights were lower ($P < 0.05$) in TR compared to Sed rats. Liver TAG were similar between TR and Sed rats whereas plasma FFA levels were lower ($P < 0.01$) in TR rats. Training was associated with higher 18:1/18:0 ratio ($P < 0.01$) in both d iets, but especially when refed the high CHO diet.
***Conclusions*:** The present data confirm the effects of exercise training on body composition. The increase in the desaturation index suggests an upregulation of hepatic SCD-1 activity with training. The absence of effect of training in liver TAG accumulation in presence of an increased SCD -1 activity suggests a higher VLDL production as an adaptation to exercise training. Supported by grants from NSERC and CIHR

T1:PO.107

PPARgamma antagonist GW9662 modulates specifically PPARgamma2 expression to abrogate differentiation of primary human preadipocyte

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Background: Rosiglitazone (RTZ) treatment enhances insulin sensitivity by activating PPAR (peroxisome proliferator and activated receptors γ) isoforms. This is accompanied by a remodelling of the adipose tissue (AT) and an increase in fat storage. We hypothesized that RTZ acts more specifically on PPARγ_2 isoform. In order to investigate the role of PPARγ_1 and PPARγ_2 during preadipocyte differentiation into mature cells capable of lipid storage, we analysed primary human preadipocytes treated with PPAR antagonist GW9662.
Methods: Preadipocytes were derived from subcutaneous and visceral biopsies of AT obtained from obese women (mean of BMI of 43±1.5 kg/m^2 (range 39.7–54.8)) during gastric by-pass surgery. Subsequently, cells were treated with serum-free medium containing dexamethasone and RTZ (DR) and supplemented or not with GW9662. They were then used to perform oil-red O staining and gene expression measurements by real-time RT-PCR (LightCycler®technology).
Results: DR treatment induces preadipocyte differentiation by enhancing PPARγ_1 and PPARγ_2 gene expression. Interestingly, GW9662 is able to prevent gene expression of mature adipocyte markers (ap2, LPL and leptin) and lipid droplet accumulation in preadipocytes of subcutaneous and visceral origin. Concomitantly, GW9662 inhibits the DR-dependent increase of PPARγ_2 expression in both SAT and VAT (by 97% and 92% respectively, P<0.001; n=7). However, it has no significant effect on PPARγ_1 expression (SAT: -55%, P=0.08; VAT: +10%, P=0.56; n=7).
Conclusions: GW9662 treatment abrogated RTZ-induced preadipocyte differentiation and lipid accumulation by inhibiting PPARγ_2 gene expression, but it did not change PPARγ_1 gene expression. These results suggest that PPARγ_2 might be the main isoform implicated in fat storage.

T1:PO.108

Normal Weight Obese syndrome: role of single nucleotide polymorphism of IL-15R and MTHFR 677C->T genes in the relationship between body composition and resting metabolic rate

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We have identified a subset of metabolically obese, but normal weight individuals, with potentially increased risks of developing the metabolic syndrome, despite their normal body mass index. We determined the relationship among body fat distribution, resting metabolic rate (RMR), total body water amount (%TBW), selected gene polymorphism on interleukin-15 receptor-alpha (IL-15R) and methylenetetrahydrofolate reductase 677C→T MTHFR 677C→T, to distinguish normal weight obese (NWO) from nonobese with a normal metabolic profile and obese individuals. We analysed anthropometric variables, body composition by Dual energy X-ray Absorptiometry (DXA), RMR by indirect calorimetry, %TBW by bioimpedence analysis (BIA), MTHFR 677C→T and IL- 15Rα genotypes of 128 clinically healthy Caucasian individuals. We compared a group of female, defined as NWO and characterised by a BMI ≤25 kg/m^2 and FM ≥30% with groups of others female, and males, represented by nonobese with a BMI ≤25 kg/m^2 and FM ≥30%, and preobese-obese individuals with BMI ≤25 kg/m^2 and %FM ≥30%; none of the males was classified as NWO. Significant correlations were found among body fat mass distribution, metabolic variables, percentage of total body water distribution and selected genetic variations. The variables that contributed significantly to the separation of classes were body tissue (Tissue), %TBW, RMR, the volumes of both oxygen (VO2) and carbon dioxide (VCO2). The distribution of MTHFR677C→T and IL-15 genotypes was significantly different between classes. Our data highlight that NWO individuals showed a significant relationship between the decrease in the basal metabolism (RMR), body fat mass increasing and total water amount. Possession of wild type homozygotes genotypes regarding IL-15 cytokine and MTHFR enzyme characterised NWO individuals.

T1:PO.109

Atrial Natriuretic Peptide (ANP) inhibits human visceral adipocytes growth via cGMP and renin-angiotensin system regulation: a new physiological pathway with clinical implications

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Visceral adipose tissue (VAT) affects both metabolism and the cardiovascular system. On the other hand, ANP and angiotensin II (AngII) influence adipose tissue: ANP induces lipolysis through cGMP, whereas AngII seems to inhibit preadipocytes differentiation. These peptides might also regulate VAT growth and primary cultures of human differentiated visceral preadipocytes (D VPA) appear to be a good model to study this aspect. We studied ANP (and 8-Br-cGMP) or AngII effects on both DVPA proliferation and angiotensinogen (AGT), AT1 receptor, and renin (REN) gene expression. Primary visceral adipocyte cultures from both human omental and perirenal tissue (n=84) were treated to differentiate (assessed by Oil Red O staining and anti –perilipin antibodies), and after serum starvation were treated for 24 hours with increasing concentrations of AngII, ANP, and 8-Br-cGMP to evaluate proliferation (by cell count, MTT, and BrDU), and AGT, AT1 and REN gene expression (by RealTime-PCR). Similar experiments were also performed with mature adipocytes. DVPA are able to divide as documented by photography. Cell proliferation was progressively inhibited by ANP (already at 10 -13M) and stimu lated by AngII treatment (starting at 10-11M). A significant AGT down - regulation, and AT1 and REN up - regulation of gene expression was observed with ANP and 8-Br-cGMP. DVPA proliferation was inhibited by ANP and stimulated by AngII; the inhibition of DVPA AGT expression by ANP -cGMP suggests reduced AngII synthesis with AT1 and REN up-regulation by lower negative feedback. If these effects occur in vivo, ANP, through cGMP and down -regulation of AGT, might inhibit VAT expansion with beneficial metabolic and c ardiovascular consequences. 877.

T1:PO.110

Prevalence of TCF4 gene microsatellite alleles in obese hypertensive patients

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Obesity is increasing worldwide together with its companions hypertension and type 2 diabetes. The obese hypertensive patients are usually at high cardiovascular risk because derangements of glucose and lipids metabolism are also present. A study in 3 different populations suggested a relationship between the TCF4 gene microsatellite DG10S478 allele "X" (with more than 5 TTTC repetitions) and type 2 diabetes. This genetic marker may be especially useful to identify patients with susceptibility to diabetes in a population with high cardiovascular risk and increased incidence and prevalence of diabetes. Thus, the objectives of this study were: 1) identify the carriers of TCF4 allele X among obese hypertensives; 2) verify the prevalence of the X allele in comparison to healthy subjects. We studied 131 obese hypertensives without diabetes, and 146 healthy subjects as control population. Genotyping of the microsatellite was performed by PCR and direct sequencing. The allelic frequencies were similar (allele X = 37.4%) to those found in the previous published study on 3 different population. We didn't find a higher allele X frequency in obese hypertensives compared to the control group (39.7% vs. 35.3%, P=0.323). Furthermore, there were no allele X-related differences in BMI and waist among groups (P=0.76). We conclude that, although TCF4 X allele could be useful to identify obese hypertensives that might develop diabetes, its prevalence is not increased in this population. Thus, accordingly to the previous published work, allele X associates with type 2 diabetes through mechanisms not linked to obesity and related consequences.

T1:PO.111

Effects of PGC-1¥á on endothelial function and apoptosis

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Aims: Central obesity is associated with increased cardiovascular morbidity and mortality. It has been proposed that increased lipid accumulation in vascular tissue and the consequent increase in oxidative stress may be a missing link between obesity and atherosclerosis. The peroxisome proliferators -activated receptor (PPAR) –gamma coactivator 1 -alpha (PGC-1alpha) is a transcriptional coactivator playing an important role in energy met abolism. PGC -1alpha is present in vascular cells, but its role in vascular endothelial cells has not been established. In this study, we examined the effect of adenoviral overexpression of PGC-1alpha (Ad-PGC-1alpha) in human aortic endothelial cells (HAECs) on apoptosis induced by linoleic acid (LA).
Methods: Effect of PGC-1 on HAECs apoptosis was evaluated by ELISA, WST -1 assay, and caspase activity. Using Ad -PGC-1 and ANT-1 siRNA, effect of PGC-1 and ANT-1 on reactive oxygen species (ROS) production, fa tty acid oxidation (FAO) and mitochondrial membrane potential (mtMP) were analyzed.
Results: PGC-1alpha prevented LA-induced endothelial apoptosis. PGC -1alpha also reduced LA-induced increases of antioxidant enzyme expression and ROS accumulation at basal state. LA decreased the activity of adenosine nucleotide translocase (ANT), and increased mtMP. In the Ad-PGC-1alpha-infected HAECs, activity and the mRNA expression of ANT -1 were increased and LA did not increase mtMP. siRNA against ANT-1 reversed the cha nges induced by PGC -1alpha.
Conclusion: These data suggest that PGC -1alpha functions as a physiologic regulator of ROS generation in endothelial cells and that part of this effect is medi ated by ANTdependent increase in FAO.

T1:PO.112

Emotionallity of mice selectively bred for high wheel-running activity

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Our previous research showed that female mice selectively bred for high voluntary wheel running activity are resistant against high fat diet-induced obesity, despite increased high fat intake in these mice relative to female controls . Since high fat feeding relates to brain serotonin levels and mood regulation, w e investigated whether selected mice 1) have altered diet selection when given a choice of fat and carbohydrates, 2) have different perfomances in behavioral tests (i.e., plus maze, open field, complex maze), and 3) have differences in biogenic amine levels in discrete brain regions relative to controls. When given choice, selected mice strongly preferred high carbohydrate diet over the fat diet, irrespective of the regular diet (i.e., chow or high fat food). Secondly, selected animals had a higher level of anxiety, as ev idenced by higher closed –arm occupation in the plus maze relative to cont rols, but selected animals were more explorative in open field and complex maze. Finally, levels of serotonin, but not of the 5HT metabolite 5HIA, were markedly suppressed in prefrontale cort ices of selected females and males, an effect that was more pronounced in animals feeding the high fat diet than those feeding chow. The results indicate that low brain ser otinin levels in high activity mice may underlie reduced fat selection and their increased state of anxiety. They do not explain, however, the higher level of spontaneous activity and explorative bevaviour in these selected mice.

T1:PO.113

Rosiglitazone reduce macrophage and chemokine expression but increase chemokine receptor expression in human adipose tissue *in vivo*

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Visceral obesity is a chronic low-grade inflammatory state associated with insulin resistance, type 2 diabetes, and cardiovascular disease. Human adipose tissue (AT) seems to be involved in the abovementioned deleterious health effects of obesity through the production of inflammatory proteins. Rosiglitazone is a PPAR -agonist with known anti-diabetic effects and reported anti-inflammatory effects. Aim of the study was to investigate the long-term effects of Rosiglitazone (4mg daily) on ATmRNA levels of macrophage specific markers [CD14, CD68], chemokines, and chemokine receptors in six abdominally obese male subjects (mean age: 50.2 ± 2.9 yrs, mean BMI: 29.3 ± 1.0 kg/m^2, mean waist: 98.7 ± 1.2 cm). AT-biopsies were obtained from the subcutaneous abdominalAT-depot at baseline, after 3, and 6 months, at which time AT-mRNA l evels were quantified using a real time RT-PCR method. Rosiglitazone reduced mRNA levels of CD14 (P<0.05), CD68 (P<0.01), MCP-1 (P<0.01), MIP-1 (P<0.05), and IL-8 non-significant (P=0.06) but increased mRNA levels of the equivalent chemokine receptors: CCR2 (P<0.05), CXC2 (P<0.05), and CXC1 non-significant (P=0.07). In conclusion, Rosiglitazone was for the first time found to exert anti-inflammatory effects in human AT *in vivo*, reducing mRNA levels of macrophage specific markers [CD14, CD68] and various chemokines. In parallel, increasing mRNA levels of the equivalent chemokine receptors were found. This suggests, that a complex interaction may exist between AT-inflammation and the need for chemokines to attract cell-types involved in tissue homeostasis [e.g. macrophages, leucocytes].

T1:PO.114

Leptin-induced Ca2+ transport in neutrophils of obese patients

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Background: The imbalance of [Ca2+]i homeostasis and free radical generation play role in the pathomechanism of athe rosclerosis in both hypercholesterolemic (HC) and obese (Ob) patients.
Objectives: To investigate [Ca 2+]i homeostasis in neutrophils of obese patients after leptin stimulation, in comparison with cells of healthy subjects (C) and there of hypercholesterolemic patients.
Design: Studies were carried out on neutrophils of 24 h ealthy control subjects, 21 Ob men and 19 lean, male patients with newly diagnosed HC.
Methods: The 100 ng/ml leptin-triggered superoxide anion, inositolt risphosphate (IP3) and Ca2+ signals were studied in neutrophils in the p resence of different inhibitors of signal pathways. The in vitro treatment of cells, with pe rtussis toxin (PTX), Ca2+-free medium, containing verapamil (V), and fluvastatin (Flu) presents an opportunity to evaluate Ca 2+ balance in Ob-neutrophils.
Results: 1**.)** The basal [Ca2+]i was higher in neutrophils of Ob - and HC-neutrophils than in control resting cells. 2.) The leptin-induced Ca2+ signal originated from the extracellular medium, and was higher than in control cells. The delayed return of [Ca2+]I to the basal level after leptin -stimulus is related to the leptin-triggered enhancement of superoxide anion generation both in Ob and HC neutrophil s. 3.) In control neutrophils the pertussis toxin sensitive Ins(1,4,5)P3 si gnal was followed by Ca2+ release from the intracellular pools.
Conclusion: In obesity, similarly to HC, the Ca2+ signal in neutrophils after leptin stimulation originated from extracellular medium, and the subsequent normalization of [Ca2+]i was delayed, as a consequence of increased amounts of leptin -triggered, statin - inhibitable superoxide anion generation. Research relating to this abstract was funded by Hungarian Health Science Council (ETT 243/2006).

T1:PO.115

The concentration-dependent biphasic effect of leptin on the cholesterol biosynthesis in human monocytes

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The aim of present study was to elucidate, whether s imilar to the c ytokines, leptin has enhancing effect on endogenous cholesterol synthesis through the upregulation of the sterol regulating element binding protein (SREBP) cleavage activating protein (SCAP) synthesis, or not. The in vitro effect of leptin was studied on human monocytes obtained from 20 healthy controls (C), and 21 patients whith hypercholester olemia (HC). The superoxide anion generation and the [14C] acetate incorporation int o the cholesterol fraction was determined, also after preincubation of monocytes with different inhibiting drugs. According to our results: 1. In the signal pathway of leptin-stimulation, the following factors were involved:Ins (1,4,5)P3 and Ca 2+ signals, the phosphatidil inositol - 3 kinase (PI3K) and the mitogen activated kinase (MAPK) cascades and the mevalonate cycle. 2. Leptin was able to induce, in both C and HC monocytes, superoxide anion generation and an enhancement in cholesterol synthesis, however, the increase was greater in HC than in control cells. 3. The Fluvastatin (Flu) -inhibitable supe roxide anion and cholesterol productions was significantly higher in both resting and leptin –stimulated HC-monocytes than in control cells. 4. Leptin has a concentration-dependent biphasic effect on cholesterol synthesis in both C and HC monocytes. The stimulation was inhibited with fluvastatin (Flu), wortmannin (WTM), and PD98059, whereas the suppression was abolished by H -7 and, in part, diminished with WTM. For the leptin induced increased cholesterol biosynthesis, the PI 3K/Akt and MAPK/ERK pathways, whereas the PKC activation for the suppression are responsible. Research relating to this abstract was funded by OTKA Hungary (K63025).

T1:PO.116

Secretory products from adipocytes mediate cell cycle progression in HT29 cells

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Among many other complications, obesity is also associated with an elevated risk for several cancer diseases, e.g. colon cancer. Adipose tissue is an active endocrine organ which secretes potential mitogenic factors like leptin. In general, MAP-kinases, especially ERK is activated by growth factors and plays a key role in cell proliferation. An increased cell proliferation in the colon is considered to represent an early event in colon carcinogenesis. Aim of the study was to investigate whether adipocytes mediate the proliferative capacity of HT29, a human colon adenocarcinoma cell line, and to elucidate which molecular mechanisms are underlying this effect. Therefore, infranatants of adipocyte cultures from different donors were prepared and the effects of those conditioned adipocyte media (CAM) on HT29 cell growth were measured by MTT assay. Cell cycle progression and apoptosis rate of cells were analysed by flow cytometry after CAM-treatment. Additionally, MAP-kinase activation was measured by a bead-based multi-plex assay. CAM mediated proliferative activity to HT29 although a heterogeneous pattern on cell growth was observed. Furthermore, CAM caused changes in cell cycle distribution with a shift from the G1 into S-phase. This effect could be mimicked by leptin (1nM). Furthermore, CAM resulted in a phosphorylation of ERK, whereas p38 and JNK were not significantly affected. This study revealed a mitogenic activity of CAM in HT29 cells. This growth stimulation was paralleled by a cell cycle shift into the S-phase. The effect seems to be mediated by an ERK-dependent pathway.

T1:PO.117

Gene expression of microfibrillar associated protein 5 (MFAP5) is downregulated after long-term weight reduction in adipose tissue - the Genobin study

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MFAP5 is a protein associated with microfibrils in the extracellular matrix, and it participates also in activation of Notch signalling pathway. Its role in the development of obesity or insulin resistance is not known. Our aim was to examine the effect of weight reduction on the *MFAP5* mRNA expression in adipose tissue (AT). Obese subjects (BMI 28-40 kg/m^2) with impaired glucose tolerance, aged 60±7 years (mean±SD) were randomized to weight reduction (WR) (N=28) or control (N=18) groups according to gender, age and BMI. Circulating glucose and insulin concentrations and subcutaneous AT biopsies were performed before and after the 33-week intervention. Gene expression was studied using quantitative PCR. *MFAP5* mRNA expression differed between the study groups (P=0.007) showing decrease (94.2±45.0 to 81.7±41.7 AU, P=0.014) in WR group. Age was significant predictor for change in MFAP5 expression. When dividing the subjects by median age (61 yrs), positive associations with *cyclin D2* (r=0.562, P=0.008; r=0.686, P=0.0001), *ADAM12* (r=0.627, P=0.002; r=0.673, P=0.001) and *PPAR 2* (r=0.562, P=0.008; r=0.606, P=0.003) mRNA expression were found in younger age group before and after the intervention, respectively. In older age group, *MFAP5* correlated negatively with serum adiponectin (r=-0.505, P=0.014; r=-0.580, P=0.004) and positively with fasting serum insulin (r=0.577, P=0.005; r=0.460, P=0.031) and leptin (r=0.426, P=0.043; r= 0.512, P=0.013) mRNA expression before and after the intervention, respectively. According to the gene expression data, *MFAP5* is highly expressed in adipose tissue. This is the first time showing that *MFAP5* expression is associated with weight reduction, and with markers of insulin resistance.
Research relating to this abstract was funded by the Academy of Finland (nr 211034; nr 211497), the Finnish Foundation for Diabetes Research, and the Sigrid Juselius Foundation.

T1:PO.118

Genetic variation in the melanocortin 3 receptor gene (MC3R): association with obesity in Chilean families

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Introduction: Melanocortin 3 rece ptor gene (MC3R) is expressed in hipothalamic areas related to energy homeostasis. Linkage studies in humans and results from animal models suggest a role for this gene in the development of excessive weight gain. Moreover, different genetic variants in M C3R have been previously associated with obesity in children and adolescents.
Aim: to assess the association between genetic polymorphisms in MC3R with the obesity and food intake behaviour in Chilean families.
Subjects and Methods: We have selected 90 tri os composed by obese children (6 –12 years-old; BMI above p95 according to NCHS/CDC 2000) and their parents. The genotypes of MC3R Thr6Lys, Val81Ile and +2138InsCAGACC were determined by means of PCR-RFLP techniques. Eating behaviour was examined through th e Three- Factor Eating Questionnaire (TFEQ -R18) in parents. The T ransmission Disequilibrium Test (TDT) was computed to assess genot ype-obesity associations.
Results: In obese children, we have detected strong linkage disequilibrium between Thr6Lys and Val81 Ile genetic variants. The preliminary frequencies of both Thr6 and Ile81 alleles were estimated as 4.9%. The preliminary frequency of the insertion +2138InsCAGACC in obese children was estimated as 14%. TDT analysis did not detect significant deviations from expected transmission under the null hypothesis of no association. No significant gender-specific differences were found in TFEQ -R18 scores in parents according to genetic categories defined by MC3R genotypes.
Conclusion: There are no sufficient evidences to support a role for MC3R variants in childhood obesity in Chilean case – parent trios. Likewise, no association between eating behaviour scores and MC3R genotypes was detected. Research supported by FONDECYT 1061096.

T1:PO.119

Plasma visfatin concentrations in morbidly obese subjects are increased after intestinal by-pass

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Background: Adipose tissue has recently been shown to s ecrete a peptide, visfatin, which is high in obese persons and in persons with type 2 diabetes mellitus (DM2). The aim of this study was t o determine the course of plasma visfatin in morbidly obese persons following weight loss after bariatric surgery in relation with the glycemia concentration.
Methods: Plasma levels of visfatin and leptin were studied in 53 morbidly obese persons (MO) before and seven months after bariatric surgery, and in 28 hea lthy persons. All the patients underwent bariatric surgery with mixed techniques: biliopancreatic diversion or gastric bypass.
Results: The presurgery levels of visfatin in the MO were greater than in the controls (55.9±39.9 vs. 42.9±16.6 ng/mL, P=0.024). This increase was due to the MO with impaired fasting glucose (IFG) (63.4±36.6 ng/mL) and with diabetes (60.0±46.0 ng/mL). The MO with normal fasting glucose had similar levels of visfatin (32.1±17.6 ng/mL) to the controls. Seven months after surgery, visfatin levels were significantly increased (84.8±32.8 ng/mL, P<0.001). This increase was independent of the presurgical glucose levels. The type of bariatric surgery had no influence on visfatin levels. Post –surgical visfatin was significantly correlated with the percentage reduction in hip measurement, leptin and the change in plasma leptin concentrations (presurgery leptin – post-surgery leptin).
Conclusion: Plasma visfatin in MO were increased, but only when accompanied by high glucose levels, even in the range of IFG. Bariatric surgery causes an increase in visfatin that is not wholly independent of the changes in the distribution of body fat. Research relating to this abstract was funded by the Instituto de Salud Carlos III (CP04/00133).

T1:PO.120

Effects of short-term food deprivation on the mRNA expression of leptin-related genes in the hypothalamus

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Leptin exerts important effects on food intake and body weight regulation by interacting with hypothalamic neurons and has a main role during starvation. The aim of the present study was to characterize changes in the expression of the principal signaling isoform of the leptin receptor (Ob-Rb) and other hypothalamic genes involved in the anorexic effects of leptin, at different hours after food deprivation, and their relation with circulating leptin levels in the rat. Adult male Wistar rats were studied under *ad libitum* feeding conditions and after 4-, 8-, or 24-h fasting. Circulating leptin levels (by ELISA) and the hypothalamic mRNA expression levels of Ob-Rb, NPY, POMC, CART, MC4-R and SOCS-3 (by RT-qPCR) were determined. Circulating leptin levels decreased as a consequence of fasting and the decrease was already significant at 4 h. In parallel to the decrease in circulating leptin, we found, in the hypothalamus, increased NPY mRNA levels, with a transient peak at 4-h fasting, and a delayed increase of Ob-Rb mRNA levels after 8-h fasting. The expression of POMC, CART, MC4-R and SOCS-3 did not significantly change during the period studied. Thus, increased expression of NPY, which is an important orexigenic neuropeptide regulated by leptin, is a rapid adaptive response associated with the lower leptin signalling during food deprivation. The expression of Ob-Rb is sensitive to the nutritional status with a relatively delayed up-regulation during food deprivation that may help buffering the anorexigenic stimulus by increasing the hypothalamic sensitivity to leptin.

T1:PO.121

Prenatal exposure to a high fat diet changes leptin-responsive gene expression in adult

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High fat consumption during pregnancy disturbs the fetal environment and may result in developmental adaptations that lead to adult metabolic dysfunction. Rat dams were fed a high fat diet (45%) prior to mating, pregnancy, and lactation. Male offspring from high fat-fed dams were weaned to control diet (HFC) or weaned to high fat diet (HFF). All animals were fasted for 24 hours be fore sacrifice at 120 days. Radioimmunoassay was used to measure serum hormones. Real -time RT-PCR was used to an alyze hypothalamic expression of key leptin -responsive genes. Leptin receptor (ObR), the down stream signal transducer and activator of transcription (STAT3), and a gene inhibited by leptin, neuropeptide Y (NPY), were measured. Expression of ObR was significantly higher in HFC (3-fold increase F(1,16)=10.07;P=0.004) and HFF (four-fold increase F(1,14)=36.28;P=0.0005) compared to control offspring. STAT3 mRNA was also significantly higher in HF C (2.6 fold increase F (1,16)=10.29, P=0.004) and HFF (3.7 fold increase F(1,14)=55.04, P<0.0005) compared to controls. N o significant difference was detected in ObR and STA T3 expression when comparing HFC to HF F offspring. Hypothalamic NPY expression was sign ificantly higher in both the HF C (1.8 fold increase F (1,16)=7.08, P=0.013) and HFF offspring (3.5 fold increase F(1,14)=41.41, P<0.0005) compared to control animals. Expression of NPY, which st imulates food intake, was highest in the adult HFF animals. Moreover, HFF animals had significantly increased body weight and ser um leptin compared to either HFC or control offspring. Thus, early life exposure to high fat is associated with persistent changes in the expression of hypothalamic components regulating food intake.

T1:PO.122

An investigation of *in vitro* release of a novel atherosclerosis associated adipokines from adipose tissue in obese patients

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Background: Antagonism of RANTES (Regulated upon Activation Normal T-cell Expressed and Secreted) receptors results in reduction of atherosclerotic plaque formation in mice. This study investigates RANTES and MCP-1 (Monocyte Chemoattractant Protein-1) release from human adipose tissue from three different sites.
Method: Subcutaneous (Sc), omental (Om) and gastric fat pad (GFP) adipose tissue samples were taken in obese patients undergoing surgery (n=9, Mean age = 43, Mean BMI = 45). RANTES, MCP-1 and leptin circulating serum levels and *in vitro* release from 24 hr adipose tissue explant levels were measured with ELISA. Body fat content was measured using bioelectrical impedance.
Results: RANTES (Sc mean = 363.3 pg/ml, Om mean = 219.8 pg/ml, GFP mean = 526.3 pg/ml), MCP-1 (Sc mean = 25 ng/ml, Om mean= 34 ng/ml, GFP mean = 22.3 ng/ml) and leptin (Sc mean = 5.7 ng/ml, Om mean =1.9 ng/ml, GFP = 10.6 ng/ml) are released from adipose tissue. There is no correlation between % body fat and serum RANTES or MCP-1 as there is with serum leptin. Unlike leptin where there is significant difference between Sc and Om release (P = 0.02), there is no significant site specific variation in MCP-1 and RANTES release *in vitro*.
Conclusion: RANTES and MCP-1 are detected in the circulation and released locally from adipose tissue. Although in this small number of patients we have not demonstrated significant site specific differences in the levels of adipokines, there may be a trend towards higher RANTES levels in adipocytes obtained from gastric fat pad.

T1:PO.123

Genotype - phenotype of CB1 receptor polymorphism in obese women

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The endocanabinoid system constitutes a central regulator of feeding behaviour and energy metabolism, and its activity depends on binding with CB1 receptors in the brain and peripheral tissues, including adipocytes. There have been no previous reports evaluating CB1 receptor polymorphisms in obese patients. The aim of study was to assess the distribution and genotype-phenotype interaction of CB1 polymorphism (1359G/A) in women with abdominal obesity (AO) in comparison with normal-weight, healthy women (NW). The study was performed on 157 AO aged 19-46 yrs and 76 age-matched NW. We evaluated: weight, height, body mass index (BMI), parameters of body composition, lipid profiles, homeostasis model of assessment (HOMA), adipokines (leptin and adiponectin serum levels), hormonal profiles (cortisol, GH and IGF-1), and serum acylated ghrelin concentration. CB1 receptor polymorphism was assessed by PCR-RSLP. Genotype distribution, and G and A allele frequency did not differ between study groups (71.3% and 28.7 in OB; 76.3% and 23.7% in NW, respectively). Amongst subjects with GG, GA and AA genotypes we found significant differences in BMI and HOMA in NW, as well as in weight, waist circumference and LDL-cholesterol in AO. An unexpected result was a significant difference in serum acylated ghrelin level in both groups. In conclusion, our results showed no differences in genotype and allele distribution in CB1 receptor polymorphism between AO-W and NW-W, but we suggest that AA donors of 1359G/A CB1 receptor polymorphism are more prone to control the feeding behaviour. These findings need to be confirmed in further studies.

T1:PO.125

Chemical derivatives of docosahexaenoic acid prevent glucose intolerance in mice fed a high-fat diet

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Polyunsaturated fatty acids of n-3 series (n-3 PUFA), especially eicosapentaenoic and docosahexaenoic (DHA) acids, act as natural hypolipidemics and reduce risk of cardiovascular disease. In rodents, n-3 PUFA also prevent obesity and insulin resistance induced by a high-fat diet. In this report, we tested the capacity of four different DHAderivatives to prevent obesity and glucose intolerance in C57BL/6N mice fed a high-fat diet. Male, 3-mo-old C57BL/6N mice were fed a high-fat (~ 35% wt/wt) diet for 4 mo. In some groups (n = 8), 1.5% of dietary lipids was replaced by DHA-derivatives (PRB-1, PRB-2, PRB-5, and PRB-7) or DHA alone. The high-fat feeding resulted in a weight gain of 25.5 ± 0.5 g. Except for PRB-5, all other DHA-derivatives significantly decreased weight gain. Especially PRB-2 exerted a very dramatic body weight-reducing effect (6.4 ± 0.8 g), but it also lowered average food intake by ~12%, while other DHA-derivatives had no effect. PRB-2 significantly improved glucose tolerance along with a reduction of post-prandial plasma insulin (also lowered by PRB-5), perhaps reflecting improved insulin sensitivity. Glucose tolerance was worsened with DHA administration. Moreover, PRB-2 reduced fasted blood glucose and p lasma lipids. Further beneficial effects of PRB- 2 on lipid content and gene expression in tissues, as well as on plasma levels of adipokines were also apparent . Chemical DHA-derivatives showed different capacities to affect various metabolic phenotypes associated with a high-fat feeding. PRB-2 had the most dramatic beneficial effect, however a part of its effect is possibly associated with a decreased caloric intake.

T1:PO.126

Different propensity to dietary obesity in C57BL/6J and A/J mice: a role for muscle non-shivering thermogenesis

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C57BL/6J and A/J inbred strains of mice are known to differ in their susceptibility to obesity induced by a high-fat (HF) diet. While C57BL/6J mice are prone to obesity, A/J mice are obesity-resistant. Our aim was to seek other possible mechanisms, besides UCP1-mediated thermogenesis, that may contribute to these strain -specific differences in propensity to obesity. Mice were born and maintained at 30oC to minimize brown-fat thermogenesis. At 4 wk of age, animals were randomly weaned onto a standard chow (ST) or HF (fat ~35% wt/wt) diet. After 2 wk, animals were subjected to *in vivo* measurements or sacrificed for the analysis of adipokines and gene expression by qRTPCR. Acute cold exposure (4 °C) resulted in a fatal decrease of deep body temperature in ST-fed A/J mice, while it was well tolerated in their HF-fed counterparts. This protective effect of HF diet in cold-exposed A/J mice was associated with higher levels of adipose tissue UCP1, however the induction of muscle thermogenesis by HF diet was also involved, as revealed by stimulation of oxygen consumption in *m. soleus*. This effect was not observed in C57BL/6J mice. This strain-specific response to HF diet was also revealed at the level of O2 consumption measured by indirect calorimetry and it was associated with a high induction of leptin in plasma of A/J mice. Thus, muscle nonshivering thermogenesis may affect propensity to dietary obesity in mice, while leptin and its ability to activate AMPK, a key sensor and regulator of cellular energy metabolism, may be involved.

T1:PO.127

Respiratory uncoupling in white fat increases whole body lipid oxidation

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In spite of low contribution of white adipose tissue (WAT) to whole-body energy expenditure, respiratory uncoupling in WAT mitochondria by ectopic expression of mitochondrial uncoupling protein 1 (UCP1) protected transgenic mice (aP2-*Ucp1* mice) against obesity (*Kopecky et al, JCI, 1995*). In this report, we studied the effect of respiratory uncoupling in WAT on whole body energy expenditure. Adult aP2-*Ucp1* mice and their nontransgenic littermates on C57BL/6J background fed chow diet *ad libitum* and maintained at 30 °C were used. Whole body O_2 consumption, CO_2 production, and deep body temperature were analyzed in resting animals, using an indirect calorimetry system INCA (Somedic; Sweden) and telemetry system E-Mitter (Mini Mitter Co. Inc.; USA). The oxygen consumption was 2-fold higher when measured at 20 °C than at 30 °C and it was not affected by the transgene. Values of CO^2/O^2 ratio (RQ value) were higher in the nontransgenic than in aP2-*Ucp1* mice, both at 20 °C 0.883 ±0.016 vs. 0.847 ±0.017) and at 30 °C (0.941 ±0.015 vs. 0.918 ±0.027). In accordance with the known atrophy of brown adipose tissue in the aP2-*Ucp1* mice, minimum deep body temperature (in °C) was lower in the transgenic mice than in nontransgenic mice if measured at 20 °C (32.7 ±0.4 vs. 34.3 ±0.2) but not at 30 °C (34.7 ±0.7 vs. 34.8 ±0.2). Modulation of WAT metabolism by ectopic UCP1 increases *in situ* lipid oxidation could be detected at the whole organism level by the indirect calorimetry. This contributes to the obesity-resistant phenotype.

T1:PO.128

Increased glucose tolerance despite low adiponectin levels in obesity-resistant aP2-Ucp1 transgenic mice fed a high-fat diet

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Transgenic mice with ectopic expression of mitochondrial uncoupling protein 1 (UCP1) in white fat (aP2-*Ucp1* mice) are resistant to a high-fat (HF) diet induced obesity. Their major phenotypic features also include reduced subcutaneous fat depots, low plasma triacylglycerols and brown fat atrophy. Here we studied the mechanisms involved in the control of glucose homeostasis in the aP2-*Ucp1* mice. Male transgenic and their C57BL/6J (B6) littermate controls were fed a HF diet (60% calories as fat) from 3 mo of age. The aP2-*Ucp1* mice were more glucose tolerant compared to controls after 2 mo of HF feeding. Despite similar blood glucose levels, plasma insulin was lower in the transgenic than in control mice. Surprisingly, insulin-sensitizing hormone adiponectin was also lower in the transgenics, which might reflect increased expression of macrophage chemoattractant protein-1 (MCP-1) and depressed expression of PPARγ in adipose tissue of these mice. At the same time, lipid content in the skeletal muscle was decreased, while the expression of glucose transporter-4 gene was increased in the transgenic mice fed a HF diet for 2 mo. However, insulin sensitivity assessed by hyperinsulinemic-euglycemic clamps in transgenic and control mice showed a similar impairment of whole-body glucose uptake after 3 wk on HF diet. In conlusion, transgenic UCP1 protects against glucose intolerance and down-regulation of *Glut4* in skeletal muscle induced by a prolonged HF feeding. This effect appears to be through improvement of lipid metabolism, independent of adiponectin. Supported in part by the grant #303/03/H065 from the Grant Agency of the Czech Republic.

T1:PO.129

The expression of eotaxin mRNA in 3T3-L1 is upregulated during the process of differentiatin

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Studies of eotaxin, in the relationship with obesity, have been reported recently. Hashimoto group reported that serum eotaxin levels are correlated with BMI. Vasudevan group also investigated that serum eotaxin levels were higher in adipose tissue of obese compared to that of lean subjects. Furthermore, we also reported that the expression of eotaxin mRNA are increased in TNF-alpha-treated human SGBS adipocyte using DNA microarray. We assumed that eotaxin may be a critical molecule that links obesity and inflammation. We investigated the expression levels of eotaxin mRNA during 3T3-L1 adipocytes differentiation as well as in *ob/ob* mice and of LPS-treated 3T3-L1 adipocytes by real-time RT-PCR. To examine the expression levels of eotaxin in 3T3-L1 adipocyte during differentiation, 3T3-L1 adipocytes were collected from days 2 to 12 after the induction of differentiation. The expression levels of eotaxin mRNA are increased from 1.5-folds to 80-folds to that of D-2, respectively. The expression level of eotaxin mRNA in *ob/ob* mice was increased more than 4-folds in comparison with lean mice. We also examined the expression level of eotaxin mRNA in 100ng/ml LPS-treated 3T3-L1 adipocyte. We expected the increased level of eotaxin by LPS treatment, but, to our surprise, its expression is decreased 40%. Our other results are well corresponds with the data of other groups. However, in our previous study, mRNA level of eotaxin was increased in TNF-alpha-treated human SGBS cells. Therefore, we need to study further about the effect of pro-inflammatory drugs on 3T3-L1 adipocytes.

T1:PO.130

Role of beta-endorphin in obesity

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Biocorect+ is six different products, containing propolis, multi-flavour honey, bee pollen, different Bulgarian herbs and lot of biological active substances. The products are designed for obese persons, who beside the overweight suffered from arterial hypertension, dislipidemia, arthrosis, holelithiasis and persons who are exposed to psycho-emotional stress.

The aim of this study was to investigate the possible positive effect of Biocorect + on different metabolic and blood parameters in obese subjects. 446 persons (200 males and 246 females) were examined. The mean age was 40, 3 years and mean body mass index (BMI) -35, 2 kg/m². After allergy examination the food (Biocorect+) was prescribed as follows: 5 days-3 x 40 drops 15 minutes before eating; 2 days 2x 60 drops (at 10 a.m. and 4p.m. in a cup of tea). At the beginning, at the end of first and second month anthropometric indexes and blood parameters (blood sugar, serum lipids and atherogenic index) were examined. After two months a significant reduction of mean weight and waist circumference was observed (mean weight-8 kg, waist circumference- 8 sm., P<0, 09). Lean body mass/fat mass ratio was increased 1, 35 times (P<0, 09). At the end of the treatment the improvement of the psycho-tonus was detected. The decrease of plasma lipids, atherogenic index and glucose was determined. The results demonstrate that Biocorect+ exhibits a favorite healing effect in obese subjects. In addition Biocorect+ has the capacity to reduce the hyperglycemia, hypertriglyceridemia and high blood glucose. Moreover, the product did not present side effects in the patients.