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HEPADIP

Hepatic and Adipose Tissue and Functions in the Metabolic Syndrome

Instrument: Integrated Project

Thematic Priority: Life Science for Health and Life Sciences, Genomics and Biotechnology for Health

Summary and Highlights of Scientific Achievements

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Summary

The main purpose of the HEPADIP project was to elucidate the role of the adipose tissues and the liver in the development of essential components of the metabolic syndrome; the dysfunctional insulin signalling, manifest in insulin resistance, glucose intolerance and dyslipidemia, primarily in the form of elevated plasma triglycerides carried mainly with very low density lipoprotein (VLDL) cholesterol, and reduced high-density lipoprotein (HDL) cholesterol. The results of the work have extensively been published in the international scientific literature, and all papers can be easily identified in the HEPADIP website, www.hepadip.org, where also an extended summary of the core papers is deposited, highlighting what these studies have added to our knowledge. As it appears from the papers, HEPADIP has contributed to a profound expansion of our knowledge about metabolic syndrome components and at the same time led to development of novel concepts and theories providing foundations for future research in this area. Hence, HEPADIP has paved the way to innovation of novel tools to combat and manage the problems of the metabolic syndrome, which - by predisposing to type 2 diabetes, cardiovascular diseases and various cancers - is a major and still increasing public health challenge in as well as in many areas outside Europe. Although dietary calorie restriction, possibly supported by increased physical activity, is able to ameliorate the problems for some time, the difficulties in implementing and maintaining this approach make it necessary to identify and validate targets possibly suitable for development of new preventive and therapeutic modalities and new standardized diagnostic criteria.

Key topics throughout the project have been causes, mechanisms and consequences of the biological and physiological processing of fatty acids and derived compounds, especially triglycerides, of inflammatory processes and signalling within the various adipose tissue depots, and between these depots and the liver. The project was organised in five lines of research focussing primarily on the adipose tissue, on the liver, on the integrated functions of both organs in animal models and in humans, and on the clinical and genetic epidemiological aspects in human populations. Owing to the distinct differences between men and women in occurrence and manifestations of the metabolic syndrome, wherever applicable the two genders were compared. There were multiple direct interactions between research lines and between partners as well as horizontal activities dealing with novel technologies and methods.

A fundamental observation, implied in the so-called lipotoxicity theory, is that free fatty acids and the saturated ones in particular, seem to be toxic, whereas triglycerides as such appear to be biologically inert, also when deposited in liver cells. Thus, triglyceride accumulation in adipose tissues may not only be an energy reserve, but also a protective adaptation to excess nutrients. Blocking of triglyceride synthesis leads to morphologically healthy appearing, but severely dysfunctional liver, associated with accumulation of ceramides and diglycerides, which also seem to be toxic. The core contention of the lipotoxicity theory is that inability to store or metabolise excess fat induces insulin resistance and metabolic dysfunction. On balance, these processes may be counteracted by ability to expand the triglyceride deposition in adipose tissue by increasing fat cell size and formation of new fat cells, increasing beta-oxidation of fatty acids, or detoxifying them by elongation and de-saturation.

This justified intense investigations of how the body, especially the adipose tissue and the liver, handles fatty acids under various conditions, especially dietary manipulations. Detailed investigations have been carried out on the regulation of adipocyte lipolysis, re-esterification and oxidation of fatty acids, of glucose utilization, of the complex interactions between adipokine formation and secretion and cellular insulin signalling. Adipogenesis, macrophage infiltration, modulations of the extra-cellular matrix and fibrosis formation in adipose tissue have proven to play a dynamic role in adipose tissue function and handling of fatty acids. The consortium brought novel data on the recruitment of the fat-consuming brown adipose tissue during cold exposure, and on the conversion of white fat cells into brown-fat like adipocytes in humans. Using a panel of study designs and advanced techniques, some of which have been further developed, refined and validated as part of HEPADIP, the role of the liver and abdominal adipose tissue depots in vivo in humans have been elucidated.

All the investigated components of the metabolic syndrome are related to liver fat accumulation, and men have more liver fat than women. Both amount of intra-abdominal and liver fat

contributes, independently of each other, to elevated VLDL triglycerides, low HDL cholesterol, increased insulin, and hepatic insulin resistance, whereas only liver fat is related to fasting glucose and liver enzymes. Amount of liver fat can be accurately and non-invasively predicted using components of the metabolic syndrome and liver enzymes. Abdominal fat accumulation is associated with hepatic fat accumulation, also at the population level. Waist circumference adjusted for body mass index is assumed to be a suitable indicator of abdominal obesity, and Individual long-term changes in this measure is associated with corresponding changes in mortality, suggesting that abdominal fat loss is a relevant target for both prevention and treatment.

Insulin stimulates hepatic glucose uptake, which is impaired in fatty liver. Abdominally obese insulin-resistant men have, when fed, increased plasma concentrations of VLDL triglycerides. Insulin suppresses large VLDL secretion in normal livers, but fails to do so in fatty liver, explaining the increased secretion of VLDL and elevated triglycerides in blood; overproduction of VLDL is crucial, but also impaired clearance related to increased apolipoprotein CIII, induced by increased hepatic glucose, contributes. The fatty acids in these patients originate from non-systemic sources (visceral depots, hepatic stores or hepatic de novo lipogenesis), but the patients also had increased oxidation of dietary fat with increased ketogenesis, and increased detoxification of saturated fat by elongation and desaturation, which may be seen as protective adaptations. Women have a more fatty acid oxidative pattern in the fasting state than men, and after fructose ingestion, men turn on storage pathways whereas women turn on oxidation.

Hepatic steatosis and inflammation is associated with activation of inflammatory cytokine and chemokine gene expression. Macrophage infiltration in visceral fat – but not in the subcutaneous - depots is correlated with inflammation and fibrosis in the liver independent of insulin resistance. Osteopontin signalling between adipose tissue and the liver may be important. C-reactive protein, while expressed predominantly in the fatty liver, is also expressed in adipose tissue, and is correlated with the size of fat mass. In the adipose tissue, ceramides, able to induce insulin resistance, apoptosis and inflammation, were the most abnormally expressed fat compounds.

Thorough investigations of gene regulation by various types of nuclear receptors (PPAR γ , PPAR α , FXR) have played a crucial role in unravelling the pathways that may be altered in adipose tissue and liver in the metabolic syndrome. Various bioinformatics modelling methods have been developed or refined for analyses of transcriptomic, lipidomic and metabolomic profiles. These studies have been supplemented by large-scale population-based search for genetic variants of importance in determining individual predisposition to the metabolic syndrome and its components.

HEPADIP contributed to the characterization of key pathogenic mechanisms in adipose tissue and liver, and to the identification of molecular targets with the potential for further development of better diagnostic tools and more efficient intervention.

Highlights of Scientific Achievements

Obesity in general

Increased morbidity and mortality from multiple causes throughout adult life when entering it as obese

Background

- Multiple studies have shown that obesity in adults is associated with increased morbidity and subsequent increased mortality, primarily from cardiovascular diseases, diabetes, some cancers, gallstones and non-alcoholic fatty liver disease, including steatohepatitis, fibrosis and cirrhosis.
- The excess morbidity and mortality is mainly attributable to abdominal obesity, likely associated with fatty liver, rather than with the general fat accumulation in adipose tissue.
- Attenuation of the effects of obesity due to adaptations and survival of the fittest has been considered, but studies have been limited in terms of coverage of the entire adult life.

Findings

- Follow-up of a group of extremely overweight young men, identified as the most obese (corresponding to BMI \geq 31 or above the 99.5 percentile) out of 360,000 men examined at the draft boards between 1943 and 1977 in the Copenhagen area and a randomly selected control group from that study population allowed through record linkage assessment of the morbidity and mortality between age 18 and above 80 years.
- The obese groups suffered from morbidity from multiple diseases, with diabetes as the most prominent, but also including for example infectious diseases, at a constantly elevated risk without any signs of attenuation through adult life.
- The excess mortality was around 2-fold throughout life, and many different diseases contributed, and median survival time (age at which 50% are still alive) was about 8 years shorter.

Reference

- Zimmermann E, Holst C, Sørensen TIA (2011) Morbidity, Including Fatal Morbidity, throughout Life in Men Entering Adult Life as Obese. PLoS ONE 6(4): e18546
- Zimmermann E, Holst C, Sørensen TI. Lifelong doubling of mortality in men entering adult life as obese. Int J Obes (Lond). 2011 Feb 15. [Epub ahead of print]

Physical activity and *FTO* rs9939609 polymorphism association with body fat accumulation

Background

- Three independent studies have shown that variation in the fat mass and obesity-associated (*FTO*) gene associates with BMI and obesity.

Findings

- In studies of 3,856 type 2 diabetic case subjects and 4,861 normal glucose-tolerant control subjects, the minor A-allele of rs9939609 associated with type 2 diabetes.
- Among 17,162 middle-aged Danes, the A-allele associated with overweight and obesity.
- Obesity-related quantitative traits such as body weight, waist circumference, fat mass, and fasting serum leptin levels were significantly elevated in A-allele carriers.
- An interaction between the *FTO* rs9939609 genotype and physical activity was found, where physically inactive homozygous risk A-allele carriers had an increased BMI compared with homozygous T-allele carriers.

Reference

- Andreasen CH ... & Hansen T. Low physical activity accentuates the effect of the *FTO* rs9939609 polymorphism on body fat accumulation. Diabetes 2008;57:95-101

Variants near-*MC4R* and obesity-related traits

Background

- Variants downstream of the melanocortin-4 receptor gene (*MC4R*) have been reported to associate with obesity.

Findings

- The minor risk alleles of rs17782313, rs17700633, and rs12970134 were associated with BMI, waist circumference, and body weight.
- In case-control studies of obesity defined by BMI, the minor C-allele of rs17782313 was associated with overweight/obesity and obesity.
- The minor A-allele of rs17700633 was associated with overweight/obesity and obesity, and the minor A-allele of rs12970134 was also associated with overweight/obesity and obesity. rs477181, rs502933, and rs4450508 were not significantly associated with obesity in the Danish population.
- The frequency of the minor risk alleles of rs17782313 and rs12970134 was higher among patients with type 2 diabetes than among glucose-tolerant individuals; however, these borderline associations were abolished after adjustment for BMI.

Reference

- Zobel DP ... & Hansen T. Variants near-*MC4R* are associated with obesity and influence obesity-related quantitative traits in a population of middle-aged people: studies of 14,940 Danes. *Diabetes* 2009;58:757-64

Ghrelin receptor locus variation in relation to measures of obesity

Background

- The growth hormone secretagogue receptor (GHSR) is mediating hunger sensation when stimulated by its natural ligand ghrelin.

Findings

- None of the variants in *GHSR* associated with measures of obesity.
- A -151C/T promoter mutation in the *GHSR* was found in two unrelated obese patients.
- The mutation resulted in an increased transcriptional activity and introduction of a specific binding for Sp-1-like nuclear extracts relative to the wild type.
- No association with obesity in -151C/T carriers versus non-carriers could be shown.

Reference

- Gjesing AP ... & Pedersen O. Family and population-based studies of variation within the ghrelin receptor locus in relation to measures of obesity. *PLoS One* 2010;5:e10084

CTNBL1 and *FDFT1* variants and measures of obesity

Background

- A genome-wide scan in unrelated US Caucasians identified rs7001819 upstream of farnesyl-diphosphate farnesyltransferase 1 (*FDFT1*) and multiple variants within catenin (cadherin-associated protein), beta-like 1 (*CTNBL1*) to associate strongly with body mass index (BMI).
- The most significantly associating variants within *CTNBL1* including rs6013029 and rs6020846 were additionally confirmed to associate with morbid obesity in a French Caucasian case-control sample.

Findings

- Both *CTNBL1* variants associated with body weight and height.
- No association was observed with BMI and waist circumference.
- In case-control studies neither of the *CTNBL1* variants showed association with overweight, obesity or morbid obesity.
- In meta-analyses of the present and the previous study, both the rs6013029 T-allele and the rs6020846 G-allele increased the risk of developing morbid obesity.

- The *FDFT1* rs7001819 C-allele showed no association with obesity-related quantitative measures or dichotomous measures of overweight, obesity and morbid obesity.

Reference

- Andreasen CH ... & Hansen T. Studies of *CTNBL1* and *FDFT1* variants and measures of obesity: analyses of quantitative traits and case-control studies in 18,014 Danes. *BMC Med Genet* 2009;10:17

Adipose tissue functions

Defect of lipolysis in human obesity

Background

- Resistance to catecholamine-induced lipolysis in subcutaneous adipose tissue has been demonstrated in obese adults and children.
- The mobilization of fat stored in adipose tissue is mediated by lipases such as hormone-sensitive lipase (HSL).

Findings

- Comparison of obese and nonobese subjects showed that obesity was associated with a decrease in catecholamine-induced lipolysis and HSL expression in mature fat cells and in differentiated preadipocytes.
- Decreased catecholamine-induced lipolysis and low HSL expression constitute a primary defect in obesity.

Reference

- Langin D... & Arner P. Adipocyte lipases and defect of lipolysis in human obesity. *Diabetes* 2005;54:3190-7

Adipose triglyceride lipase and hormone-sensitive lipase in adipocyte lipolysis

Background

- Lipolysis is the catabolic pathway by which triglycerides are hydrolyzed into fatty acids.
- Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) have the capacity to hydrolyze in vitro the first ester bond of triglycerides, but their respective contributions to whole cell lipolysis in human adipocytes is unclear.

Findings

- Immunocytochemistry experiments revealed that acute forskolin treatment promotes HSL translocation from the cytosol to small lipid droplets and redistribution of ATGL from the cytosol and large lipid droplets to small lipid droplets, resulting in enriched colocalization of the two lipases.
- HSL or ATGL overexpression resulted in increased triglyceride-specific hydrolase capacity, but only ATGL overexpression increased whole cell lipolysis.
- HSL silencing had no effect on basal lipolysis and only partially reduced forskolin-stimulated lipolysis.
- Silencing of ATGL or its co-factor CGI-58 significantly reduced basal lipolysis and essentially abolished forskolin-stimulated lipolysis.
- ATGL/CGI-58 acts independently of HSL and precedes its action in the sequential hydrolysis of triglycerides in human hMADS adipocytes.

Reference

- Bezaire V... & Langin D. Contribution of adipose triglyceride lipase and hormone-sensitive lipase to lipolysis in hMADS adipocytes. *J Biol Chem* 2009;284:18282-91

Lipolysis and lipid mobilization in human adipose tissue

Background

- Lipolysis is under tight hormonal regulation.
- Triacylglycerol (TAG) stored in adipose tissue (AT) can be rapidly mobilized by the hydrolytic action of the three main lipases of the adipocyte.
- The released non-esterified fatty acids (NEFA) are used by other tissues during times of energy deprivation.

Conclusions

- This paper is a comprehensive review of recent progress made in understanding the mechanisms of activation of the various lipases and control by endocrine and autocrine/paracrine factors.
- The manipulation of lipolysis has therapeutic potential in the metabolic disorders frequently associated with obesity and probably in several inborn errors of metabolism.

Reference

- Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. *Prog Lipid Res* 2009;48:275-9

Expression of glycerol kinase and metabolism genes in adipocytes

Background

- Conversion of human white adipocytes into fat cells with some properties of brown adipocytes is an attractive therapeutic strategy.
- The transcriptional coactivator peroxisome proliferator-activated receptor γ (PPAR γ) coactivator 1 α (PGC-1 α) was initially described as a metabolic regulator of adaptive thermogenesis in brown adipose tissue.

Findings

- Primary cultures of human adipocytes were transduced with a PGC-1 α adenovirus and treated with PPAR γ and PPAR α agonists.
- Among the large number of genes regulated by PGC-1 α independently of PPAR γ , new targets involved in metabolism included the gene encoding glycerol kinase (GyK).
- The induction of GyK by PGC-1 α was observed at the levels of mRNA, enzymatic activity, and glycerol incorporation into triglycerides.
- PPAR α was shown to bind and activate the GyK promoter.
- The induction of GyK by PGC-1 α and PPAR α may promote a futile cycle of triglyceride hydrolysis and fatty acid re-esterification.

Reference

- Mazzucotelli A ... & Langin D. The transcriptional coactivator peroxisome proliferator activated receptor (PPAR) γ coactivator-1 α and the nuclear receptor PPAR α control the expression of glycerol kinase and metabolism genes independently of PPAR γ activation in human white adipocytes. *Diabetes*. 2007;56:2467-75

PPAR α regulation of beta-oxidation and glucose utilization in adipocytes

Background

- There is a paucity of studies regarding the role of PPAR α in human adipocytes.
- We showed that PPAR α regulates expression and activity of glycerol kinase in these cells.

Findings

- The PPAR α agonist GW7647 induced an up-regulation of beta-oxidation gene expression and increased palmitate oxidation.
- Glycolysis was strongly reduced at transcriptional and functional levels by GW7647 leading to a decrease in pyruvate and lactate production.
- PPAR α can selectively up-regulate beta-oxidation and decrease glucose utilization in human white adipocytes.

Reference

- Ribet C ... & Langin D. Peroxisome proliferator-activated receptor- α control of lipid and glucose metabolism in human white adipocytes. *Endocrinology* 2010;151:123-33

Brown adipose tissue and thermogenesis

Functional brown adipose tissue in healthy adults

Background

- Brown adipose tissue was known to be important animals and human infants for thermogenesis, but thought to be absent in adult humans

Findings

- Our studies showed first time that functional brown adipose tissue exists in supraclavicular area in adult healthy humans. This proof-of-concept findings were possible using cold-activation, positron emission tomography imaging and guided fat biopsies .
- Further examinations are ongoing to evaluate differences between lean and obese subjects and study the physiology of brown adipose tissue, its potential activators in addition to cold

Reference

- Virtanen KA ... & Nuutila P. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009;360:1518-25

Farnesoid X receptor in the regulation of adaptive thermogenesis

Background

- FXR regulates bile acid, lipid and glucose metabolism.
- A role of FXR in the regulation of adaptive thermogenesis was not identified.

Findings

- FXR has a role in adaptative thermogenesis in a leptin-dependent manner and is thus a modulator of energy homeostasis.
- FXR-KO mice do not display an alteration of energy expenditure at the basal state.
- FXR-KO mice display an accelerated entry into torpor upon fasting and are cold-intolerant.

Reference

- Cariou B ... & Staels B. FXR-deficiency confers increased susceptibility to torpor. *FEBS Lett* 2007;581:5191-8

Phosphorylation of farnesoid X receptor promotes its transcriptional activity

Background

- FXR regulates bile acid, lipid and glucose metabolism.
- It was not known whether post-translational modifications can affect FXR activities.

Findings

- FXR is phosphorylated by calcium-dependent protein kinase C (PKC).
- The inhibition of PKC impairs the regulation of FXR target genes.
- PKC modulates FXR transcriptional activities.
- Activated PKC induces the phosphorylation of FXR in hepatoma cells and PKC α phosphorylates FXR *in vitro* in the DNA-binding domain.
- The phosphorylation of FXR induces the recruitment of PGC1 α .

Reference

- Gineste R ... & Staels B. Phosphorylation of farnesoid X receptor by protein kinase C promotes its transcriptional activity. *Mol Endocrinol* 2008;22:2433-47

Brown adipocytes fighting metabolic complications in obesity

Background

- The role of white and brown adipose tissues in energy metabolism is well established.
- The existence of brown fat in adult humans was until very recently a matter of debate
- The molecular mechanisms underlying brown adipocyte development remain largely unknown.

Conclusions

- In 2009, several studies brought direct evidence for functional brown adipose tissue in adults.
- New factors involved in brown fat cell differentiation have been identified.
- Different populations of brown fat cell precursors have been identified according to the anatomical location of the fat depots: a precursor common to skeletal muscle cells and brown adipocytes from brown fat depots, and a progenitor cell common to white adipocytes and brown adipocytes that appear in certain conditions in white fat depots.
- There is mounting evidence that mature white adipocytes, including human fat cells, can be converted into brown fat-like adipocytes.
- These data open up new opportunities for the development of drugs for obesity and its metabolic and cardiovascular complications.

Reference

- Langin D. Recruitment of brown fat and conversion of white into brown adipocytes: strategies to fight the metabolic complications of obesity? *Biochim Biophys Acta* 2010;1801:372-6

Adipose tissue inflammation

Inflammatory proteins in adipose tissue

Background

- Adipose tissue secretes a number of proteins so called adipokines. Many of them are inflammatory proteins. The role of most adipokines and their regulation is unclear.
- Visceral fat accumulation is more dangerous than superficial (subcutaneous adipose tissue) fat accumulation for metabolic syndrome. This is in part due to increased delivery of fatty acids from visceral fat to liver due to high rate of adipocyte lipolysis. The mechanisms behind the increase in lipolysis are not known.

Findings

- Release of inflammatory proteins within human adipose tissue is not only important for metabolic syndrome but also for the normal function of human adipose tissue. One inflammatory protein, tumour necrosis factor alpha plays a superior regulatory role and the production of several adipose inflammatory proteins is regulated by the novel transcription factor TWIST-1.
- Endothelin is released as an adipokine from human adipose tissue. It selectively inhibits insulin action in visceral fat cells so that this depot releases more fatty acids than other fat adipose depot.

References

- Arner E, Rydén M, Arner P. Tumor necrosis factor alpha and regulation of adipose tissue. *N Engl J Med* 2010;362:1151-3
- Pettersson AT ... & Rydén M. A possible inflammatory role of twist1 in human white adipocytes. *Diabetes* 2010; 59:564-71
- van Harmelen V ... & Arner P. Vascular peptide endothelin-1 links fat accumulation with alterations of visceral adipocyte lipolysis *Diabetes* 2008;57:378-86

Interactional and functional centrality in transcriptional co-expression networks

Background

- The biological interpretation of the vast amount of transcriptomic data generated by microarray technology, remains a difficult task and is often biased by the noise embedded within the data. This comes as a consequence of the sensitive nature of this technology, differences between platforms etc.
- Few tools exist to help scientists analyze and interpret this kind of data and new original developments are needed.

Findings

- Our work consisted on investigating and developing new original ways to “amplify” the biological signal and lower the noise within the transcriptomic data. We based our research on the graph theory and integrated biological knowledge stored and structured in public databases such as KEGG and Gene Ontology to gene expression data.
- We developed a two-layer model that helps identifying functional modules (biological themes and genes in functional and co-expression networks). These algorithms were implemented in an R-package named FunNet who became available as a publically web tool (<http://funnet.info>).
- A more recent development consisted in imagining an original way to quantify the functional importance of genes within the co-expression network. We showed that the conventional topological centrality measures such as degree and betweenness were not adapted to co-expression networks and proposed a new integrated measure names “annotation theme centrality” that was also implemented into FunNet.

Reference

- Prifti,E ... & Henegar C. FunNet: an integrative tool for exploring transcriptional interactions. *Bioinformatics* 2008;24:2636-8
- Prifti E ... & Henegar C. Interactional and functional centrality in transcriptional co-expression networks. *Bioinformatics* 2010; 26:3083-9

Adipose tissue macrophages and adipocytes gene regulation during dietary weight loss and maintenance

Background

- Moderate weight loss improves insulin sensitivity and many of the concurrent medical complications associated with obesity such as type 2 diabetes
- The long-term outcome of dietary interventions remains poorly understood because of a lack of knowledge regarding the kinetics of complex adipose tissue adaptations during weight loss and weight maintenance and its relation with insulin sensitivity.

Findings

- Transcriptome profiling during a dietary intervention program composed of an energy restriction phase with a 4-week very-low-calorie diet and a weight stabilization period composed of a 2-month low-calorie diet followed by 3-4 months of a weight maintenance diet revealed two main patterns of variations.
- The first involved 464 mostly adipocyte genes involved in metabolism that were downregulated during energy restriction, upregulated during weight stabilization, and unchanged during the dietary intervention.
- The second comprised 511 mainly macrophage genes involved in inflammatory pathways that were not changed or upregulated during energy restriction and downregulated during weight stabilization and dietary intervention.
- Adipose tissue macrophages and adipocytes show distinct patterns of gene regulation and association with insulin sensitivity during the various phases of a dietary weight loss program.

Reference

- Capel F ... & Langin D. Macrophages and adipocytes in human obesity: adipose tissue gene expression and insulin sensitivity during calorie restriction and weight stabilization. *Diabetes* 2009;58:1558-67

Adipokines and dietary interventions in human obesity

Background

- Obesity is characterized as a condition of low-grade inflammation with altered adipose tissue function and secretion of various adipokines.
- Dietary interventions could modulate cytokine levels in a favourable way.

Conclusions

- This paper is a systematic review of studies performed in the last 13 years investigating dietary intervention programmes accompanied with weight loss in relation to profile of adipokines.
- Dietary interventions leading to 5-10% weight loss modulate production of certain adipokines and generally induce improvement of insulin sensitivity.
- The amelioration of obesity complications is not coherent with the pattern of adipokine regulation, except maybe for leptin.
- Global analysis of the adipose tissue secretome and measurement of panels of adipokines may prove more informative than studies on individual molecules.

Reference

- Klimcakova E ... & Langin D. Adipokines and dietary interventions in human obesity. *Obes Rev* 2010;11:446-56

Subcutaneous and visceral adipose tissue metabolism-related and immune response-related gene expression

Background

- It is not known whether biological differences reported between sc adipose tissue (SAT) and visceral adipose tissue (VAT) depots underlie the pathogenicity of visceral fat.
- We compared SAT and VAT gene expression according to obesity, visceral fat accumulation, insulin resistance, and presence of the metabolic syndrome.

Findings

- Considering the two fat depots together, 1125 genes were more and 1025 genes were less expressed in lean compared with metabolic syndrome subjects.
- Functional annotation clustering showed, from lean to metabolic syndrome subjects, progressive down-regulation of metabolic pathways including branched-chain amino acid, fatty acid, carbohydrate, and mitochondrial energy metabolism and up-regulation of immune response genes involved in toll-like receptor, TNF, nuclear factor- κ B, and apoptosis pathways.
- Metabolism and immune response genes showed an opposite correlation with fat mass, fat distribution, or insulin resistance indices.
- The increase in adiposity and the worsening of metabolic status are associated with a coordinated down-regulation of metabolism-related and up-regulation of immune response-related gene expression. Molecular adaptations in SAT prove as discriminating as those in VAT.

Reference

- Klimcakova E ... & Langin D. Worsening of Obesity and Metabolic Status Yields Similar Molecular Adaptations in Human Subcutaneous and Visceral Adipose Tissue: Decreased Metabolism and Increased Immune Response. *J Clin Endocrinol Metab* 2011;96:E73-E82

Subcutaneous and omental adipose tissue fibrosis and metabolic alterations

Background

- Investigations performed in mice and humans have acknowledged obesity as a low-grade inflammatory disease. Several molecular mechanisms have been convincingly shown to be involved in activating inflammatory processes and altering cell composition in white adipose

tissue (WAT). However, the overall importance of these alterations, and their long-term impact on the metabolic functions of the WAT and on its morphology, remain unclear.

- The composition of subcutaneous (scWAT) and omental WAT (oWAT) fibrosis in obesity and its relationship with metabolic alterations and surgery-induced weight loss are still unknown.

Findings

- The functional profiles and transcriptomic interactions characterizing the adipose tissue of subjects in the obese static phase confirm the strong relationship linking inflammatory processes and ECM remodeling, associated with different inflammatory cell types and to some degree of interstitial fibrosis in WAT.
- Type I and III collagens were more frequently observed in fibrous bundles, whereas type VI collagen surrounded parenchymal adipocytes, particularly in obese subjects.
- The negative relationship between the amount of oWAT fibrosis and adipocyte size, on the one hand, and circulating triglycerides, on the other hand. Multivariate analysis showed that these factors are closely related, suggesting that omental fibrosis influences the circulating triglycerides level by limiting adipocyte size.
- Negative correlation between the percentage of total fibrosis in scWAT and the percentage of fat mass loss in patients after gastric bypass surgery: both high levels of scWAT fibrosis and systemic inflammation influenced the individual capacity to lose fat mass.
- Our work provides deeper knowledge on the characterization of adipose depots and enlightens the importance of remodeling.

Reference

- Henegar C ... & Clement K. Adipose tissue transcriptomic signature highlights the pathological relevance of extracellular matrix in human obesity. *Genome Biol* 2008;9:R14
- Divoux A ... & Clément K. Fibrosis in human adipose tissue: composition, distribution, and link with lipid metabolism and fat mass loss. *Diabetes* 2010;59:2817-25

Lipid partitioning between adipocytes and tissue macrophages and lipotoxicity

Background

- Obesity-associated insulin resistance is characterized by a state of chronic, low-grade inflammation that is associated with the accumulation of M1 proinflammatory macrophages in adipose tissue.
- It was unknown the mechanisms leading to this accumulation and polarisation.

Findings

- Our data indicate that the M1 ATM polarization in obesity might be a macrophage-specific manifestation of a more general lipotoxic pathogenic mechanism.
- This indicates that strategies to optimize fat deposition and repartitioning toward adipocytes might improve insulin sensitivity by preventing ATM lipotoxicity and M1 polarization

Reference

- Prieur X ... & Vidal-Puig A. Differential Lipid Partitioning Between Adipocytes and Tissue Macrophages Modulates Macrophage Lipotoxicity and M2/M1 Polarization in Obese Mice. *Diabetes* 2011;60:797-809

Insulin signalling in adipose tissue

Farnesoid X receptor regulates adipocyte function and differentiation and peripheral insulin sensitivity

Background

- FXR regulates hepatic glucose metabolism.
- A function of FXR in the regulation of peripheral glucose metabolism was not identified.
- A function of FXR in peripheral tissues was not identified.

Findings

- FXR regulates peripheral insulin sensitivity.
- FXR regulates adipocyte function and differentiation by promoting PPAR γ and interfering with the Wnt/ β -catenin pathways.
- FXR-KO mice display decreased adipose tissue mass and peripheral (and not hepatic) insulin resistance.
- FXR expression is detectable at low level in adipose tissue ; it is modulated by high fat diet and induced during adipocyte differentiation *in vitro*.
- Mouse embryonic fibroblasts (MEFs) isolated from FXR-KO mice display altered adipocyte differentiation, even upon PPAR γ activation.
- FXR-KO *ob/ob* mice display resistance to PPAR γ treatment.
- The Wnt/ β -catenin pathway and target genes are more expressed in FXR-KO adipose tissue and MEFs.

References

- Cariou B ... & Staels B. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. *J Biol Chem* 2006;281:11039-49
- Abdelkarim M ... & Staels B. The farnesoid X receptor regulates adipocyte differentiation and function by promoting peroxisome proliferator-activated receptor-gamma and interfering with the WNT/ β catenin pathways. *J Biol Chem* 2010;285:36759-67

Retinol-binding protein 4 unrelated to insulin sensitivity during calorie restriction

Background

- Retinol-binding protein 4 (RBP4) is an adipokine that may play a role in the development of insulin resistance.
- Its regulation had not been investigated during multiple phase weight loss programs (e.g., an energy restriction phase with a 4-week very-low-calorie diet and a weight stabilization period composed of a 2-month low-calorie diet followed by 3-4 months of a weight maintenance diet)

Findings

- Glucose disposal rate assessed with the euglycemic hyperinsulinemic clamp increased during very-low-calorie diet and remained elevated thereafter.
- Plasma levels of RBP4 decreased after very-low-calorie diet and, although increasing at subsequent phases, remained lower than prediet values.
- Adipose tissue mRNA levels were diminished after very-low-calorie diet, and increased during low-calorie diet and weight maintenance to reach basal values.
- Basal RBP4 levels or diet-induced variations of RBP4 were not different in lean women and two groups of obese women with high- and low-insulin sensitivity.
- The study does not bring evidence for a role for RBP4 in the regulation of diet-induced changes in insulin sensitivity.

Reference

- Vitkova M ... & Langin D. Plasma levels and adipose tissue messenger ribonucleic acid expression of retinol-binding protein 4 are reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity. *J Clin Endocrinol Metab* 2007;92:2330-5

Insulin cross-talks with IL-6 signalling in adipocytes

Background

- IL-6 signalling has previously been demonstrated to negatively affect insulin signaling and, thus, reduce insulin sensitivity.

Findings

- Our studies have revealed that insulin cross-talks with IL-6 signalling in adipocytes and exerts an anti-inflammatory effect by attenuating IL-6-induced inflammation. Insulin reduced the activation of key molecules in the IL-6 signalling pathway and, consequently, reduced also transcription of IL-6-regulated pro-inflammatory genes.

- Further examination of the IL-6 signalling pathway revealed PKC- δ as an important molecule and critical for a functional IL-6 signalling in adipocytes. Inhibition of PKC- δ function or expression attenuated the IL-6 signalling transduction as well as the transcription and secretion of IL-6-regulated pro-inflammatory markers.

Reference

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- Wallerstedt E ... & Andersson CX. Protein kinase C- δ is involved in the inflammatory effect of IL-6 in mouse adipose cells. *Diabetologia* 2010;53: 946-54

Hypoxia as a new mechanism in insulin resistance in adipose tissue

Background

- Hypoxia could play a role in adipose tissue dysfunction in obesity.

Findings

- Hypoxia inhibited insulin signaling and effect in cultured adipocytes.
- This inhibition was reversible under normoxic conditions
- HIF1 was involved in these effects
- Redd1, an inhibitor of the mTOR pathway that is induced by hypoxia is also induced by hyperinsulinemia through HIF1 and the PI3Kinase/mTOR pathway
- Emerging concept: Hypoxia could be envisioned as a new mechanism that participates in insulin resistance in adipose tissue of obese patients.

References

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- Regazzetti C, Bost F, Le Marchand-Brustel Y, Tanti JF, Giorgetti-Peraldi S: Insulin induces REDD1 expression through hypoxia-inducible factor 1 activation in adipocytes. *J Biol Chem* 2010;285:5157-64

Inflammatory processes modulate insulin pathway and GLUT4 trafficking

Background

- Adipose tissue plays a central role in the control of glucose and lipid metabolism.
- Several kinases such as the JNK kinases are involved in insulin resistance
- ERK kinases activation is deleterious in insulin signaling in obese humans and mice.
- The serine phosphorylation of IRS1 plays a role in insulin resistance
- Inflammatory stresses are likely to participate in insulin resistance

Findings

- IL-1 β activates ERK and blunts insulin effects through a decrease in the expression of Insulin Receptor Substrate-1 (IRS-1) both in murine and human adipocytes.
- The invalidation of ERK1 in *ob/ob* mice rendered the mice more insulin sensitive without modifying their obesity. This was linked with a decreased expression of inflammatory markers in the adipose tissue and an improvement in its function.
- Tpl2 was identified as a new inflammatory kinase selectively involved in inflammatory cytokine-induced ERK activation and in cytokines-induced lipolysis and down-regulation of insulin signaling. in both murine and human adipocytes
- Tpl2 mRNA expression was up-regulated in adipose tissue of obese mice and patients and correlated with TNF- α expression, was increased by chronic-treatment of adipocytes with inflammatory cytokines
- The level of sortilin mRNA and protein, which is involved in insulin stimulated glucose transport was decreased in adipose tissue from obese mice and humans, in correlation with an increased TNF- α expression.
- Rab4a was identified as a small GTPase involved in glucose transport, and its level was modified in adipose tissue of obese subjects.

- **Emerging concept:** Chronic low-grade inflammation in obesity could contribute to insulin resistance by modulating insulin pathway and the expression of proteins that control GLUT4 trafficking such as sortilin and Rab4b. We propose that Tpl2/ERK pathway is a new actor in adipose tissue inflammation and dysfunction in obesity, opening the possibility of new therapeutic targeting.

References

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- Kaddai V ... & Cormont M. Rab4b is a small GTPase involved in the control of the glucose transporter GLUT4 localization in adipocyte. *PLoS One* 2009;4:e5257

Drugable enzyme involved in lipid signalling and expressed specifically in visceral adipose tissue, critical for obesity, inflammation and insulin resistance

The target has been identified, validated and further developed by the Genfit company, but is still unpublished.

Select target from candidate proteins applying scientific, medical and technical criteria

Background

- The target that was chosen by Genfit is enzymatic protein that plays important role in lipid signalling
- Some rudimentary data were published on Target polymorphism in human
- Target is expressed at low level in diverse tissues and cell-types

Findings

- Genfit has demonstrated that this target is preferentially expressed in the visceral adipose tissue in men as opposed to the subcutaneous adipose tissue
- Genfit has demonstrated that the expression of the target correlates with the expression of inflammatory markers, in the visceral adipose tissue of obese and diabetic subjects but not in healthy subjects

Validation of potential targets in critical experiments in *vitro* and in *vivo*

Background

- Target is involved in lipid signalling

Findings

- Genfit demonstrated that target overexpression in preadipocytes stimulates adipocyte differentiation whereas its pharmacological inhibition results in decreased adipocytic differentiation, as judged from lipid accumulation and specific marker expression
- Target deficient animals were resistant to high fat induced obesity, retained normal insulin sensitivity and were protected from inflammatory infiltration in the adipose tissue

Preparation of a “drug target package” for decision of further development

Background

- It was known that target is druggable, since non-specific inhibitors have already been described
- It was not known what are the physiological consequences of a specific inhibition of that target

Findings

- Genfit has developed and validated HTS-ready screening assay to screening campaign for inhibitors of that target
- Genfit has developed a whole array of profiling assays (cell-free and in vitro) to enable hit to lead studies
- Genfit has identified several putative target inhibitors by existing patent analysis and addressed the selectivity and the potency of these compounds with profiling assays developed in this program
- Genfit demonstrated the technical feasibility of target selective inhibition

Adipose tissue expandability and lipotoxicity

Bioinformatics strategies for lipidomics analysis

Background

- Lipids are an important and highly diverse class of molecules having structural, energy storage and signaling roles.
- Modern analytical technologies afford screening of many lipid molecular species in parallel.
- One of the biggest challenges of lipidomics is elucidation of important pathobiological phenomena from the integration of the large amounts of new data becoming available.

Findings

- Computational and informatics approaches were developed to study lipid molecular profiles in the context of known metabolic pathways and established pathophysiological responses, utilizing information obtained from modern analytical technologies.
- The lipid pathway reconstruction methodology facilitates identification and interpretation of high-throughput lipidomics data.
- The utility of our approach was demonstrated by its application to the lipidomic characterization of the fatty liver of the genetically obese insulin resistant ob/ob mouse model.
- We identified the parallel associations between the elevated triacylglycerol levels and the ceramides, as well as the putative activated ceramide-synthesis pathways of relevance to understanding lipotoxicity in the liver.

Reference

- Yetukuri L ... & Orešič M. Bioinformatics strategies for lipidomics analysis: characterization of obesity related hepatic steatosis. BMC Systems Biology 2007;1:e12

Ectopic triglyceride accumulation as protective adaptation to excess of nutrients

Background

- Accumulation of triglycerides in liver and skeletal muscle is a marker of insulin resistance in the context of the metabolic syndrome.
- That there are lipid species such as DAGs and ceramides that are associated with insulin resistance.

Findings

- The concept that lipid quality is as important or more than the total amount of lipids.
- Data coming from our POKO and ob/ob mice indicated that accumulation of triglycerides may be a protective adaptation to accommodate the excess of nutrients resulting from increased

nutrient flux and that probably should be interpreted more as a sign of overnutrition than a pathogenic mechanism leading to insulin resistance and metabolic complications.

- In fact blockade of triglyceride biosynthesis happen to produce morphologically more “healthy” livers, but results in enrichment of ceramides and severe metabolic alteration. Conversely, there is now evidence that facilitated triglyceride formation results in depletion of more reactive lipid species and improved metabolism.
- Systems biology studies of the livers of the Ob/ob reinforced the concept of the relevance of lipid quality by showing that there is a correlation between triglycerides and ceramides. In this respect the levels of triglycerides can be used as markers of the other lipid species and have prognostic and diagnostic value.
- However the slope of this correlation can change and we can have paradoxical examples of healthy livers enriched in triglycerides and unhealthy livers depleted of triglycerides. In this regard this concept has diagnostic, prognostic and also therapeutic implications if we consider its potential relevance for liver transplant viability.

References

- Medina-Gomez G ... & Vidal-Puig A. PPAR γ 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. *PLoS Genet* 2007; **3**:e64
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- Yetukuri L ... & Oresic M. Bioinformatics strategies for lipidomics analysis: characterization of obesity related hepatic steatosis. *BMC Syst Biol* 2007;1:12

Adipose tissue expandability, lipotoxicity and the metabolic syndrome

Background

- Obesity and the amount of adipose tissue is epidemiologically correlated with obesity associated complications

Findings

- Here we propose that the absolute fat mass of an individual does not determine how likely this individual is to develop metabolic disturbances. Rather, the key determinant leading to insulin resistance and related complications is the inability to continue to store excess energy appropriately in adipose tissue. This is the hypothesis of the exhausted adipose tissue expandability as a pathogenic mechanism linking obesity, lipotoxicity and its metabolic complications.
- This obviously is a change in the accepted paradigm that has therapeutic implications from the point of view of the determinants of maximal expansion, predictive diagnostic and therapeutic markers

Reference

- Virtue S, Vidal-Puig A. It's not how fat you are, it's what you do with it that counts. *PLoS Biol* 2008;6:e237
- Vidal-Puig A, Unger RH. Special issue on lipotoxicity. *Biochim Biophys Acta* 2010;1801:207-8
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- Medina-Gomez G ... & Vidal-Puig A. PPAR γ 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. *PLoS Genet* 2007; **3**:e64
- Gray SL ... & Vidal-Puig A. Leptin deficiency unmasks the deleterious effects of impaired peroxisome proliferator-activated receptor- γ function (P465L PPAR γ) in mice. *Diabetes* 2006;55:2669-77

Allostasis to the pathogenesis of the metabolic syndrome

Background

- The concept of allostasis is a concept borrowed from the psychosocial model of social stress, that has not been applied to the understanding of the metabolic syndrome and therapeutic approaches.

Findings

- We successfully applied the concept of allostasis to the pathogenesis of the metabolic syndrome and its therapeutic approaches.
- **Allostasis** is defined as maintenance of normality at the expense of changes, that per se particularly when maintained for a long period of time may cause metabolic stress and by itself cause disease.
- We observed that the apparent normality of PPAR γ murine models was associated with important molecular adaptations not only in white adipose tissue, but very remarkably in brown fat. These molecular adaptations include activation of previously unknown alternative pathways related to prostaglandin metabolism, which seems to contribute to maintain energy homeostasis despite strong genetic challenges.
- This in our opinion is important because has the potential to a) use these mechanisms as diagnostic/predictors of metabolic stress and increased susceptibility to disease and b) offer promise for potential alternative therapeutic targets, which actually may represent some of the adaptive mechanisms that prevents successful therapeutic outcomes particularly common when treating obesity.

Reference

- Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome--an allostatic perspective. *Biochim Biophys Acta* 2010;1801:338-49
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Abdominal obesity and fatty liver

Co-occurrence of abdominal obesity and fatty liver

Background

- The reason for differences in fat accumulation in the liver among persons who do not abuse alcohol is unknown
- It has been hypothesized that visceral fat releases free fatty acids and adipokines and thereby exposes the liver to fat accumulation

Findings

- Human studies with data on abdominal fat and liver fat were reviewed
- The reviewed studies suggest that abdominal fat is positively associated with liver fat probably accounted for by visceral fat

Reference

- Jakobsen MU ... & Overvad K. Abdominal obesity and fatty liver. *Epidemiol Rev* 2007;29:77-87

Relative contributions of intra-abdominal and liver fat to metabolic syndrome

Background

- Abdominally obese individuals with the metabolic syndrome often have excess fat deposition both intra-abdominally (IA) and in the liver
- The relative contribution of these two deposits to variation in components of the metabolic syndrome was unclear

Findings

- IA fat and liver fat contents were correlated ($r=0.65$)
- Both IA fat and liver fat independently of each other and of gender, age, subcutaneous fat and lean body mass explain variation in serum triglyceride, HDL cholesterol, insulin concentrations and hepatic and adipose tissue insulin sensitivity
- Liver fat, but not IA fat, independently predicted glucose and liver enzymes.
- Subcutaneous fat, but not liver fat and IA fat, explained variation in blood pressure
- Both IA fat and liver fat depots are important independent predictors of the dyslipidaemia, reduced insulin sensitivity and disturbed glucose homeostasis in the metabolic syndrome.

Reference

- Kotronen A ... & Sørensen TIA. Comparison of Relative Contributions of the Intra-Abdominal and Liver Fat to Components of the Metabolic Syndrome. *Obesity (Silver Spring)* 2010;19:23-8

Liver fat are related to components of the metabolic syndrome independent of obesity

Background

- Subjects with increased liver fat due to non-alcoholic causes were known to be insulin resistant but it was unknown how the individual components of the metabolic syndrome are related to liver fat measured by ¹H-MRS in a large number of subjects and whether such relationships were dependent upon gender and independent of obesity.

Findings

- All components of liver fat are related to components of the metabolic syndrome independent of obesity. Men have more liver fat for any given body mass index.

Reference

- Kotronen A ... & Yki-Järvinen H. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* 2007;92:3490-3497

Adipokine effects on glucose metabolism in cultured liver cells

Background

- Adipose tissue is an active secretory (endocrine) organ. Obesity is commonly associated with alterations in glucose metabolism in the liver
- Which factors are able to affect parameters of glucose metabolism in cultured liver cells? Which of them might be derived from adipose tissue and could therefore explain the close association between an elevated fat mass and NASH/NAFLD?

Findings

- Short term (6hrs) and long term (30hrs) incubation with defined adipokines exerted different responses for most of the metabolic readouts under investigation (TNF-alpha, MCP-1, Angiotensin II, IL-6, SDF1- α , PAI-1, RANTES).
- RANTES showed the most consistent effects on gluconeogenesis in rat FAO hepatoma cells
- Both basal and cAMP/dexamethasone stimulated gluconeogenesis was reduced upon chronic incubation with RANTES

- In addition, mRNA levels of key enzymes of gluconeogenesis (PEPCK, G6Pase) were negatively affected by chronic incubation with RANTES in FAO cells. This effect was also dose-dependent.
- Less pronounced and heterogeneous effects were also observed with the chemokine SDF1-alpha. Whereas incubation with SDF1- α (400ng/ml) resulted in a reduction of cAMP/dexamethasone stimulated gluconeogenesis, mRNA levels of PEPCK were significantly up-regulated at the same time.
- Angiotensin II, resulting from angiotensinogen expression in visceral adipose tissue, caused a stimulation of cAMP/dexamethasone stimulated gluconeogenesis, whereas PEPCK-mRNA levels were reduced.

Reference

- [Hans Hauner/Thomas Skurk Manuscript in preparation]

Farnesoid X receptor modulates hepatic carbohydrate metabolism

Background

- The nuclear receptor Farnesoid X Receptor (FXR) is expressed in the liver.
- A function of FXR in the regulation of hepatic glucose metabolism was not identified.

Findings

- FXR regulates hepatic glucose metabolism.
- FXR-deficient (FXR-KO) mice display an accelerated response to high carbohydrate refeeding (accelerated induction of glycolytic and lipogenic genes and accelerated repression of gluconeogenic genes).
- FXR-KO mice display no alteration of hepatic insulin sensitivity.
- FXR represses glucose-induced glycolytic (LPK) and lipogenic (ACC1, Spot14) gene expression in *in vitro* mouse hepatocyte models.

References

- Duran-Sandoval D ... & Staels B. The farnesoid X receptor modulates hepatic carbohydrate metabolism during the fasting-refeeding transition. J Biol Chem 2005;280:29971-9

Visceral adipose tissue and inflammation correlate with elevated liver tests

Background

- The severity of fatty liver is positively correlated with visceral adipose tissue (VAT) and insulin resistance in both obese and non-obese individuals.
- Levels of high-sensitive C-reactive protein (hs-CRP), a marker of low-grade inflammation, have been linked to visceral adiposity.

Findings

- In overweight and obese patients, liver tests, especially ALT and GGT, are associated with VAT. After correction for BMI and hs-CRP, ALT and GGT are significantly higher in patients with increased VAT.
- Analyzing different levels of ALT and GGT and their possible relationship with VAT, hs-CRP and insulin resistance (estimated using homeostasis model assessment HOMA-IR) were significantly different between low-normal levels and high-normal (borderline) levels of ALT and GGT, suggesting it may be appropriate to lower the upper limit of normal (ULN) of these liver tests.
- Multiple regression analysis showed that each liver test has its own most important independent determinant(s). For AST this was VAT, for ALT and ALP this was HOMA-IR, for GGT this were triglycerides. Adding hs-CRP to the model showed that besides the metabolic and anthropometric variables, inflammation has an important influence (except for GGT for which VAT and triglycerides remain the most important determinants).

Reference

- Verrijken A ... & Van Gaal L. Visceral adipose tissue and inflammation correlate with elevated liver tests in a cohort of overweight and obese patients. Int J Obes 2010;34:899-907

Chemerin and fatty liver in morbidly obese before and after bariatric surgery

Background

- Chemerin is a new adipokine involved in *in vitro* adipogenesis and insulin resistance and associates with body mass index (BMI) *in vivo*.
- The role of chemerin in morbid obesity and associated metabolic diseases and postsurgery-induced weight loss is still unclear.

Findings

- Chemerin concentrations are elevated in morbidly obese patients and correlated with insulin resistance and with several markers of liver injury.
- Surgical obesity treatment inducing strong weight, fat mass loss, and improvement of hepatic function, decreases circulating chemerin levels in a more prolonged way than other adipokines as leptin.

Reference

- Sell H ... & Clément K. Chemerin correlates with markers for fatty liver in morbidly obese patients and strongly decreases after weight loss induced by bariatric surgery. *J Clin Endocrinol Metab* 2010;95:2892-6

Peroxisomal and microsomal lipid pathways in hepatic steatosis and pro-inflammatory state

Background

- Hepatic steatosis is associated with obesity and the metabolic syndrome.
- Accumulation of fat in liver is considered to be secondary to excess accumulation of fat in adipose tissue.
- Adipose tissue inflammation and development of insulin resistance leads to transfer of free fatty acids and to peripheral tissues, including liver cells.
- Besides this adipocentric view, inflammation at the liver level may also induce peripheral insulin resistance and susceptibility to diet-induced obesity.
- In addition, these pathways for obesity, inflammation and insulin resistance are influenced by the genetic make-up of each individual.
- The impact of the genetic background is unknown.

Findings

- Comparison of liver transcriptomes of mice with different genetic backgrounds (A/J and C57Bl/6) and susceptibilities to diet-induced hepatosteatosis was performed after different periods of normal chow or high fat diet feeding.
- Quantitative RT-PCR analysis, western blotting, and biochemical measurements were used to confirm transcriptomic data.
- Liver lipidomic analysis was performed at the same time points as the transcriptomic analysis.
- Two lipid metabolic pathways were associated with resistance to diet-induced hepatic steatosis: increased peroxisomal beta-oxidation (10 genes, including the L-bifunctional enzyme, *Ehhadh*) and microsomal elongase (*Elovl5*) and desaturases (*Fads1*, *Fads2*).
- Increased microsomal enzymes can detoxify saturated fat by converting them into unsaturated fatty acids.
- They also convert n-6 essential fatty acids into arachidonic acid, which accumulates as 2-arachidonylglycerol (2-AG), a cannabinoid receptor agonist.
- 2-AG binding to CB2 receptors on Kupffer cells reduces the production of cytokines (IL-1 and G-CSF) and, consequently liver and plasma pro-inflammatory state.
- We thus identified two hepatic lipid pathways, whose activity depend on the genetic background, and which can explain the resistance to diet-induced hepatic steatosis and the reduced pro-inflammatory state of the liver.
- **Perspectives:** Assessment of *Ehhadh* variants in human cohorts show associations with different degrees of steatosis and fasting glucose levels. Assessment of the phenotype of *Ehhadh*^{-/-} and *Ehhadh*^{+/-} mice: homozygous KO mice display diet-induced liver inflammation,

fibrosis, and death due to liver failure; heterozygous KO mice show diet-induced hepatic steatosis, impaired fasting glucose and obesity.

Reference

- Hall D ... & Thorens B. Peroxisomal and microsomal lipid pathways associated with resistance to hepatic steatosis and reduced pro-inflammatory state. *J Biol Chem* 2010;285:31011-23

Lipid and glucose metabolism and fatty liver

Magnetic resonance spectroscopy measuring tissue fatty acid composition

Background

- The fatty acid composition of serum lipids has been shown to predict the metabolic syndrome, and the ectopic accumulation of fat in tissues such as the muscle and liver is also associated with the metabolic syndrome. However, in order to measure the fatty acid composition of tissue fat, invasive biopsies must be taken.
- There is a need for less invasive techniques to measure the fatty acid composition of adipose tissue, liver and muscle.

Findings

- In these studies, we used a 1.5 Tesla clinical imager and proton magnetic resonance spectroscopy to measure tissue fatty acid composition, validated against gas-chromatography. The studies represent an important methodological advancement.

References

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- Lundbom J ... & Lundbom N. Characterizing human adipose tissue lipids by long echo time 1H-MRS in vivo at 1.5 Tesla: validation by gas chromatography. *NMR Biomed* 2010;23:466-72
- Lundbom J ... & Lundbom N. Long echo time 1H-MRS suggests liver fat is more saturated than subcutaneous and visceral fat. *NMR Biomed.* 2010;24:238-45

Positron emission tomography for the quantitative studies of liver metabolism and perfusion

Background

- due to its anatomical location, the liver has been inaccessible for direct non-invasive measurements and little is about the normal regulation of liver glucose and fatty acid uptake in human

Findings

- Our studies have validated methods for the use of the positron emission tomography for the quantitative studies of liver metabolism and perfusion

Reference

- Iozzo P ... & Nuutila P. Quantification of liver glucose metabolism by positron emission tomography: validation study in pigs. *Gastroenterology* 2007;132:531-42
- Iozzo P ... & Nuutila P. Fatty Acid Metabolism in the Liver, Measured by Positron Emission Tomography, Is Increased in Obese Individuals. *Gastroenterology* 2010;139:846-56

Sex differences in lipid and glucose kinetics

Background

- Young women generally have lower plasma triglyceride (TG) concentrations and higher non-esterified fatty acid (NEFA) concentrations than men, and are more insulin-sensitive
- Men respond more than women to the TG-raising effect of dietary fructose

Findings

- After fructose ingestion, men turn on storage pathways whereas women turn on oxidation
- Women have a more oxidative pattern of fatty acid metabolism in the liver in the fasting state than do men
- Men show dilution of systemic fatty acids within the liver during the oxidation pathways, perhaps representing the effects of increased visceral or liver fat

Reference

- Tran C ... & Tappy L. Sex differences in lipid and glucose kinetics after ingestion of an acute oral fructose load. *Br J Nutr* 2010;104:1139-47
- Marinou K ... & Fielding BA. Young women partition fatty acids towards ketone body production rather than VLDL-triacylglycerol synthesis, compared to young men. *Br J Nutr* 2011;105:857-65

Insulin and energy balance regulate liver glucose and fatty acid uptake in human

Background

- Due to its anatomical location, the liver has been inaccessible for direct non-invasive measurements and little is about the normal regulation of liver glucose and fatty acid uptake in human

Findings

- Our studies have revealed that insulin stimulates hepatic glucose uptake and this effect is impaired in patients with diabetes and/or fatty liver.
- In addition to rapid decrease of liver fat content low calorie diet modifies also hepatic insulin sensitivity and decreases fatty acid uptake in liver and other organs.

References

- Viljanen AP ... & Nuutila P. Effect of Weight Loss on Liver Free Fatty Acid Uptake and Hepatic Insulin Resistance. *J Clin Endocrinol Metab* 2009;94:50-5
- Viljanen AP ... & Nuutila P. Effects of weight loss on visceral and abdominal subcutaneous adipose tissue blood-flow and insulin-mediated glucose uptake in healthy obese subjects. *Ann Med* 2008;41:152-60

Isotopically labelled glycerol for kinetic studies

Background

- Kinetic studies using stable isotopes have indicated that increased very-low density lipoprotein (VLDL)-triglyceride production occurs in people with the metabolic syndrome.
- It is important to analyse samples in a way that excludes glycerol metabolic recycling during the study period. Such methods are limited.

Findings

- The method described in this paper has provided a useful validation of the analysis of isotopically labelled glycerol (excluding metabolic recycling) for ongoing kinetic studies.

Reference

- Adiels M ... & Fielding BA. Optimization of N-methyl-N-[tert-butyldimethylsilyl]-trifluoroacetamide as a derivatization agent for determining isotopic enrichment of glycerol in very-low density lipoproteins. *Rapid Commun.Mass Spectrom* 2010;24:586-92

Splanchnic fat in VLDL and fat oxidation in insulin-resistant men

Background

- Increased liver fat, insulin resistance and increased hepatic VLDL-TG secretion are closely associated
- There is a suggestion that increased de novo lipogenesis contributes to liver fat accumulation, but little study of the pathways of hepatic fat metabolism in the fed state

Findings

- Insulin-resistant men have increased VLDL-TG concentrations in the fed state
- Compared with insulin-sensitive men, the main difference is in non-systemic sources of fatty acids (potentially visceral fat, hepatic fat stores, and hepatic de novo lipogenesis)
- Quite unexpectedly, abdominally-obese insulin resistant men were found to have increased oxidation of dietary fat (hepatic and systemic) in the fed state
- Hence greater ketogenesis is associated with greater VLDL-TG secretion, in contrast to what might be expected
- We also found increased activity of hepatic pathways to modify saturated fatty acids (elongation and desaturation) in abdominally obese insulin resistant men.

Reference

- Hodson L ... & Fielding BA. The contribution of splanchnic fat to VLDL triglyceride is greater in insulin-resistant than insulin-sensitive men and women: studies in the postprandial state. *Diabetes* 2007;56:2433-41
- Hodson L ... & Karpe F. Greater dietary fat oxidation in obese compared with lean men: an adaptive mechanism to prevent liver fat accumulation? *Am J Physiol Endocrinol Metab* 2010;299:E584-92

Adipose tissue inflammation with increased ceramides at high liver fat content

Background

- Previous studies have shown that obese insulin resistant subjects have an increased infiltration of macrophages in adipose tissue and an increased expression of chemokines and cytokines.
- We hypothesized that if a fatty liver indeed distinguishes between those with the metabolic/insulin resistance syndrome independent of obesity, then the above changes should characterize subjects with increased liver fat content compared to equally obese subjects with a normal liver fat content. We also searched for possible lipid mediators of insulin resistance by analyzing adipose tissue composition using UPLC/MS.

Findings

- Subjects with a fatty liver due to non-alcoholic causes have an increased amount of macrophages in adipose tissue and increased expression of chemokines and cytokines compared to weight-, age-, and gender matched subjects with a normal liver fat content.
- Ceramides, which can induce both insulin resistance, inflammation and apoptosis, were the most abnormally expressed lipid species in adipose tissue of subjects with a high as compared to normal liver fat content.
- These data imply that abnormalities in adipose tissue and a fatty liver are coupled independent of obesity and that ceramides could be involved in the pathogenesis of adipose tissue insulin resistance in humans.

Reference

- Kolak M ... & Yki-Järvinen H. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. *Diabetes* 2007;56:1960-8

Composition and lipid spatial distribution of high-density lipoprotein particles

Background

- Low level of high-density lipoprotein cholesterol (HDL-C) is a powerful risk factor for cardiovascular disease.
- However, despite the reported key role of apolipoproteins, specifically, apoA-I, in HDL metabolism, lipid molecular composition of HDL particles in subjects with high and low HDL-C levels is currently unknown.

Findings

- Low HDL-C subjects had elevated triacylglycerols and diminished lysophosphatidylcholines and sphingomyelins.

- Using information about the lipid composition of HDL particles in these two groups, we reconstituted HDL particles in silico by performing large-scale molecular dynamics simulations.
- In addition to confirming the measured change in particle size, we found that the changes in lipid composition also induced specific spatial distributions of lipids within the HDL particles, including a higher amount of triacylglycerols at the surface of HDL particles in low HDL-C subjects.
- Our findings have important implications for understanding HDL metabolism and function.
- The study demonstrates for the first time the power of combining molecular profiling of lipoproteins with dynamic modeling of lipoprotein structure.

Reference

- Yetukuri L ... & Orešič M. Composition and lipid spatial distribution of High Density Lipoprotein particles in subjects with low and high HDL-cholesterol. *J Lipid Res* 2010;51:2341-51

***PPAR* α Leu162Val polymorphism and obesity, type 2 diabetes, dyslipidaemia**

Background

- Peroxisome proliferator-activated receptor-alpha (*PPAR* α) is a nuclear receptor capable of regulating the expression of genes involved in peroxisomal and mitochondrial beta-oxidation pathways.
- The common Leu162Val polymorphism in the gene encoding *PPAR* α has inconsistently shown association with quantitative traits related to obesity, type 2 diabetes, and dyslipidaemia.

Findings

- We did not show any associations between diabetes and *PPAR* α genotype.
- The Leu162Val polymorphism was not associated with WHO-defined obesity or dyslipidaemia.
- Quantitative trait studies of metabolic variables showed a difference in fasting serum triglyceride concentrations among homozygous Val-carriers.
- Val/Val was associated with increased fasting serum total cholesterol concentrations.

Reference

- Sparsø T ... & Pedersen O. Relationships between the functional *PPAR* α Leu162Val polymorphism and obesity, type 2 diabetes, dyslipidaemia, and related quantitative traits in studies of 5799 middle-aged white people. *Mol Genet Metab* 2007;90:205-9

Promoter variant in hepatic lipase and HDL-cholesterol modulated by physical activity

Background

- Hepatic lipase plays a pivotal role in the metabolism of high-density lipoprotein (HDL) and low-density lipoprotein by involvement in reverse cholesterol transport and the formation of atherogenic small dense low-density lipoprotein.

Findings

- The A allele of rs2070895 associated with an increase in fasting serum HDL-cholesterol
- The allelic effect on HDL-cholesterol was modulated by interaction with self-reported physical activity because vigorous physically active homozygous A-allele carriers showed increased HDL-cholesterol compared with homozygous G-allele carriers.

Reference

- Grarup N ... & Pedersen O. The -250G>A promoter variant in hepatic lipase associates with elevated fasting serum high-density lipoprotein cholesterol modulated by interaction with physical activity in a study of 16,156 Danish subjects. *J Clin Endocrinol Metab* 2008;93:2294-9

GCKR polymorphism and serum triacylglycerol, insulinaemia, and risk of type 2 diabetes

Background

- Genome-wide association studies have suggested that a polymorphism in GCKR, the gene encoding the glucokinase regulatory protein, is involved in triacylglycerol regulation.

Findings

- The minor *GCKR* A-allele of rs780094 is associated with an increased level of fasting serum triacylglycerol, impaired fasting and OGTT-related insulin release, reduced homeostasis model assessment of insulin resistance, WHO-defined dyslipidaemia and a modestly decreased risk of type 2 diabetes.
- Significantly increased fasting serum insulin concentrations were demonstrated when analysing the *GCK -30A* and *GCKR* rs780094 G-alleles in an additive model.

Reference

- Sparsø T ... & Pedersen O. The *GCKR* rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. *Diabetologia* 2008;51:70-5

AHSG and type 2 diabetes and dyslipidemia

Background

- The gene encoding the alpha2 Heremans-Schmid glycoprotein (AHSG) is a credible biological and positional candidate gene for type 2 diabetes and the metabolic syndrome.
- Previous attempts to relate AHSG variation with type 2 diabetes and obesity in Swedish and French Caucasians have been largely successful.

Findings

- The -469T>G (rs2077119) and IVS6+98C>T (rs2518136) polymorphisms were associated with type 2 diabetes.
- Two *AHSG* haplotypes were associated with dyslipidemia.
- Indications of epistatic effects of *AHSG* variants with the *IRS1* Gly971Arg polymorphism were observed for fasting serum triglyceride concentrations.

Reference

- Andersen G ... & Pedersen O. *AHSG* tag single nucleotide polymorphisms associate with type 2 diabetes and dyslipidemia: studies of metabolic traits in 7,683 white Danish subjects. *Diabetes* 2008;57:1427-32

Utilization of in vivo analysis of triglyceride composition by magnetic resonance spectroscopy

Background

- Several developments have been made in the methodology available for these studies. A new method for analysis in vivo of adipose tissue and liver triglyceride composition by using non-invasive proton magnetic resonance spectroscopy (MRS) have been developed, validated.

Findings

- Impact of the fatty acid composition in triglycerides was studied in vitro using different oils as phantom materials to find out how different fatty acids will affect the echo time behavior and T2 relaxation both important parameters in the AMARES fitting of the data. The detailed FA composition of different oils was determined by gas chromatography. The data demonstrated that FA composition has an impact on the echo time behavior of triglyceride resonances. Long echo time spectroscopy is requested for in vivo studies to identify differences in triglyceride FA composition. These data were the backbone to develop the methodology for in vivo measurements in humans.
- MRS with various echo times was used to characterize the subcutaneous adipose tissue triglyceride composition and to validate the findings with FA analysis of the fat biopsies (n= 17

subjects) by gas chromatography (GC). The data demonstrated that long TE MRS, using a 1.5 Tesla clinical imager, provides a robust non-invasive method for characterizing adipose tissue triglycerides in vivo.

- The novel methodology was used to determine the composition of liver fat in vivo in humans. The novel finding is that long TE in MRS can be used to determine the double-bond content of the liver allowing to estimate the unsaturation of the triglyceride (DB/FA) in both adipose tissue and in the liver fat. We report that liver fat is more saturated than abdominal subcutaneous or visceral fat. This is novel and important finding that has a significant relevance for future studies to evaluate the liver fat composition in dietary studies etc.
- The methodology utilizing multiple stable isotopes to track both leucine and glycerol has been developed and validated. This allows to follow-up VLDL assembly, secretion and catabolic rate in vivo in man. The mathematic modeling and analyses of the kinetic data has established a new and more specific method to measure glycerol enrichment in VLDL-TG.
- We used multiple traces labeled with stable isotopes to study the acute suppression of large VLDL 1 secretion rate by insulin and reported that insulin down-regulates the secretion of large VLDL 1 particles in subjects with low liver fat but fails to suppress VLDL 1 secretion in subjects with high liver fat resulting in overproduction of VLDL1 reflected in the elevation of serum TG that is known to associate with liver fat. Our study highlights the pathophysiology behind the relationship between serum triglycerides and dyslipidemia with increase of liver fat.
- We studied the effects of insulin infusion on plasma endocannabinoid levels in obese and non-obese subjects followed by measurement of 2-AG and anandamine concentrations. The data suggested that insulin negatively controls plasma EC levels.
- Our studies have consistently demonstrate that overproduction of very low density lipoproteins is the hall mark of the dyslipidemia in the metabolic symptoms, type 2 diabetes and in people with high liver fat. These studies have relevance to our understanding of factors regulating lipid metabolism in the liver and furthering the new understanding of the pathogenesis of dyslipidemias and the dynamic changes associated with liver fat deposition.
- We have investigated hepatic lipoprotein metabolism in people with abdominal adiposity with and without hypertriglyceridemia to investigate why a proportion of people with excess adipose tissue remains metabolically healthy and do not develop dyslipidemia. The data demonstrated that hypertriglyceridemia associated with abdominal obesity requests a dual defect of increased hepatic TG secretion particularly of large VLDL 1 particles and impaired clearance capacity related to increase of apolipoprotein CIII.

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- Taskinen M-R ... & al. Dual metabolic defects are required to produce hypertriglyceridemia in obese subjects *ATVB* 2011;submitted

Variants of *SREBF1* and type 2 diabetes, glycemia, and insulin resistance

Background

- Previous quantitative trait associations between the sterol regulatory element-binding factor 1 gene (*SREBF1*) and type 2 diabetes have been inconclusive.

Findings

- The minor alleles of rs2297508, rs11868035, and rs1889018 associated with a modestly increased risk of type 2 diabetes.
- A meta-analysis of all published studies confirmed this association.
- The diabetes-associated alleles also associated strongly with a higher plasma glucose at 30 and 120 min and serum insulin at 120 min during an oral glucose tolerance test and the minor allele of rs1889018 with a surrogate measure of insulin sensitivity.
- The diabetes-associated alleles associated with a modestly increased A1C level in the population-based Inter99 of middle-aged subjects and in the ADDITION study of high-risk individuals.

Reference

- Grarup N ... & Pedersen O. Association of variants in the sterol regulatory element-binding factor 1 (*SREBF1*) gene with type 2 diabetes, glycemia, and insulin resistance: a study of 15,734 Danish subjects. *Diabetes* 2008;57:1136-42

***ApoCIII* gene transcription is induced by glucose in the liver**

Background

- Apolipoprotein CIII (apoCIII) is a regulator of hepatic and plasma triglyceride metabolism.
- Plasma apoCIII protein levels are elevated in type 2 diabetes patients.
- It was not known whether apoCIII levels are affected by altered glucose metabolism.

Findings

- ApoCIII gene transcription is induced by glucose in the liver and may thus represent a link between hypertriglyceridemia and hyperglycemia in type 2 diabetes.
- Glucose induces apoCIII gene transcription in primary rat hepatocytes and immortalized human hepatocytes (IHH cell line).
- The glucose-mediated induction of apoCIII involves the transcription factors ChREBP and HNF-4 α and is inhibited after activation of the nuclear receptors FXR and PPAR α , but not LXRs.
- Plasma apoCIII protein levels correlates positively with fasting plasma glucose and plasma glucose excursion after a glucose bolus in obese patients.

References

- Caron S ... & Staels B. Transcriptional Activation of Apolipoprotein CIII Expression by Glucose May Contribute to Diabetic Dyslipidemia. *Arterioscler Thromb Vasc Biol* 2011;31:513-9

Pancreatic islets cells

FXR expressed in pancreatic β -cells protects them from lipotoxicity

Background

- FXR is expressed in liver, intestine and adipose tissue where it regulates bile acid, lipid and glucose metabolism.
- A role of FXR in pancreas function was not identified.

Findings

- FXR is expressed and functional in human islets and β -cell lines.

- FXR is predominantly cytoplasmic in the islets from lean mice and nuclear in those of obese mice.
- The islets of FXR-KO mice display a normal architecture, but an alteration of islet-specific gene expression and glucose-induced insulin secretion.

Reference

- Popescu IR ... & Staels B. The nuclear receptor FXR is expressed in pancreatic β -cells and protects human islets from lipotoxicity. *FEBS Lett* 2010;584:2845-51

Polymorphism near *GAD65* and body mass index and glycaemia

Background

- The glutamate decarboxylase gene (*GAD2*) encodes *GAD65*, an enzyme catalysing the production of the gamma-aminobutyric acid (GABA), which interacts with neuropeptide Y to stimulate food intake.
- It has been suggested that in pancreatic islets, GABA serves as a functional regulator of pancreatic hormone release.
- Conflicting results have been reported concerning the potential impact of *GAD2* variation on estimates of energy metabolism.

Findings

- The G-allele associated with modestly lower BMI.
- In a case-control study of obesity, the G-allele frequency in 2582 participants with BMI < 25 kg/m² was higher compared with 968 participants having BMI \geq 30 kg/m².
- Of the 5857 subjects, GG carriers had lower fasting plasma glucose levels and lower 30-min oral glucose tolerance test (OGTT)-related plasma glucose levels adjusted for sex, age and BMI.
- Analysing subjects who were both normoglycaemic and glucose tolerant (n = 4431) GG carriers still had lower fasting plasma glucose concentrations.

Reference

- Boesgaard TW ... & Pedersen O. A -243A-->G polymorphism upstream of the gene encoding *GAD65* associates with lower levels of body mass index and glycaemia in a population-based sample of 5857 middle-aged White subjects. *Diabet Med* 2007;24:702-6

PPAR α activation protects human pancreatic islets from lipotoxicity

Background

- The nuclear receptor Peroxisome Proliferator-Activated Receptor α (PPAR α) controls lipid and glucose metabolism.
- The role of PPAR α in the development of genetic-induced obesity (*ob/ob* mice) is unknown.

Findings

- PPAR α improves the adaptative response of the pancreatic β -cell to pathological conditions.
- PPAR α -deficient mice on an *ob/ob* genetic background (PPAR α -KO *ob/ob*) display no modification of body weight and of peripheral insulin resistance compared to *ob/ob* mice, but develop an age-dependent hyperglycemia.
- PPAR α -KO *ob/ob* mice have pancreatic β -cell dysfunction (smaller islet area, decreased insulin secretion in response to glucose *in vivo* and *in vitro*).
- PPAR α activation protects human pancreatic islets from lipotoxicity.

Reference

- Lalloyer F ... & Staels B. Peroxisome Proliferator-Activated Receptor Improves Pancreatic Adaptation to Insulin Resistance in Obese Mice and Reduces Lipotoxicity in Human Islets. *Diabetes* 2006;55:1605-13

Liver injury in NAFLD

Obesity-driven macrophage accumulation determines liver fibrosis and inflammatory damage

Background

- Macrophage accumulation in omental adipose tissue is associated with liver fibro-inflammation in morbidly obese subjects
- The influence of glycemic status on the relationship between omental adipose tissue macrophages and the various histopathologic components of non-alcoholic fatty liver diseases remains unknown.

Findings

- The prevalence of fibro-inflammation appears unrelated to the degree of insulin resistance.
- Macrophage infiltration in subcutaneous adipose tissue is unrelated to liver histopathology, including steatosis.
- The number of omental macrophages significantly increases with the worsening of steatosis and this relationship is dependent on glycemic status: macrophages in omental adipose tissue are insufficient by themselves to promote fat accumulation in the liver, although it contributes to aggravate steatosis in conjunction with insulin resistance.
- Severe hepatic fibroinflammation is associated with higher numbers of macrophages in omental adipose tissue, irrespective of the degree of insulin resistance: obesity-driven macrophage accumulation is an independent determinant of liver fibrosis and inflammatory damage.

Reference

- Tordjman J ... & Clement K. Association between omental adipose tissue macrophages and liver histopathology in morbid obesity: influence of glycemic status. *J Hepatol* 2009;51:354-62

Advanced glycation end products induce reactive oxygen species in hepatic stellate cells

Background

- Metabolic syndrome patients present high concentrations of advanced glycation products (AGEs) when compared to healthy individuals.
- These molecules can bind and interact with several membrane receptors and influence several cell functions like production of reactive oxygen species (ROS), apoptosis and proliferation.
- Although patients with NAFLD have increased levels of AGEs, little is known about its effect on the progress of the disease, and specifically on hepatic stellate cells (HSCs), an important player in liver fibrosis development.

Findings

- HSCs express five AGE receptors (SR-AI, SR-BI, CD36, Galectin-3 and RAGE) at gene and protein expression, showing an upregulation of all, except SR-BI, cell during activation in vitro.
- Three different AGE types induce production of ROS in HSCs
- ROS production is dependent on NADPH oxidase, PKC δ and Rac1, as tested by chemical inhibition and siRNA knockdown.
- AGEs induce upregulation of fibrogenic genes, such as α -smooth muscle actin, procollagen 1a1 and PDGFr β .
- Together these results indicate that AGEs could play a role in the development of liver fibrosis in metabolic syndrome patients.

Reference

- Guimaraes EL ... & van Grunsven LA. Advanced glycation end products induce production of reactive oxygen species via the activation of NADPH oxidase in murine hepatic stellate cells. *J Hepatol* 2010;52:389-97

Bioinformatics identified candidate genes for non-alcoholic fatty liver disease

Background

- It has been estimated that around 20% of all adults have non-alcoholic fatty liver disease (NAFLD)
- NAFLD associates with insulin resistance and type 2 diabetes (T2D) and it has been suggested that it might predict the presence or future development of the metabolic syndrome
- While environmental factors causing NAFLD are well-known, it has been suggested that genetics factors also predispose to NAFLD and that these might explain the difference in NAFLD progression between individuals.
- Several candidate genes for NAFLD have been identified, but none of them have shown to account for the entire genetic contribution to the development of NAFLD

Findings

- EHHADH, ECHS1, HADHA, HADHB, and ACADL were identified as candidate genes by a bioinformatics approach.
- These were examined for variant associations to quantitative traits of NAFLD-related phenotypes.
- 10 nominal statistical significant associations ($P < 0.05$) to quantitative metabolic traits were identified.
- The case-control study showed associations between variation in the five genes and T2D, central obesity, and MetS, respectively.
- Bonferroni adjustments for multiple testing negated all associations.
- Using a bioinformatics approach we identified five candidate genes for NAFLD.
- We failed to provide evidence of associations with major effects between SNPs in these five genes and NAFLD-related quantitative traits, T2D, central obesity, and MetS.

Reference

- Banasik K ... & Hansen T. Bioinformatics-driven identification and examination of candidate genes for non-alcoholic fatty liver disease. *PLoS One* 2011;6:e16542

Gene pathways in progression from fatty liver to NASH

Background

- Although there are some individual proteins and genes already related with NAFLD disease, no further studies based on modern high throughput techniques have been used to identify novel targets.

Findings

- By integrating gene expression profiling of liver biopsies from NASH patients and liver samples from a mouse model of steatohepatitis (MAT1A-KO), a gene-pathway associated with NASH was identified.
- Critically, the transcription factor Sp1 was described as a hub in the NASH-gene pathway network.
- We postulated that hyperphosphorylation of Sp1 may be involved in the genesis of steatosis and that other factors such as oxidative stress may trigger its progression to NASH.
- In addition, we have been able to evaluate changes of expression in hundreds of proteins simultaneously from each of the three groups included in our study by using a proteomics analysis (DIGE combined with MALDI TOF/TOF); subjects controls, non-alcoholic steatosis, and non-alcoholic steatohepatitis. The proteins validated were further tested in serum samples of different cohorts of patients.
- Fibroblast growth factor 21 (FGF21) is a hepatic protein that plays a critical role in metabolism. We found a positive correlation between BMI and FGF21, which fell within a narrow range. There was no change in FGF21 in response to fasting or KD. Hepatic FGF21

expression was significantly elevated in NAFLD, which correlated with a substantial increase in serum FGF21. Serum FGF-21 was also increased significantly in NASH.

References

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Methione metabolism in animal models of NAFLD

Background

- Methionine metabolism and S-adenosylmethionine levels are crucial for liver functionality and have been involved in NAFLD disease.
- Animal models are important tools for understanding NAFLD disease.

Findings

- The levels of the enzyme involved in SAMe metabolism, Glycine N-methyltransferase (GNMT), is crucial for maintenance the proper equilibrium of this metabolite in the liver. An increase in SAMe levels triggers the development of NAFLD and the progression to fibrosis and HCC in a spontaneous way.
- The reduction of SAMe levels by treatment of GNMT KO animals with nicotinamide induces an increase in the activity of Nicotinamide methyltransferase, a reduction of SAMe levels and a reversion in NAFLD phenotype associated to those animals.

References

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Progression of steatosis to NASH also due to factors from adipose tissue

Background

- The modification of adipose tissue functions can directly alter the liver functions,
- Identification of makers of hepatic inflammation (NASH) is required for the diagnosis,
- Hepatic inflammation is associated with hepatocyte death,
- C-reactive protein (CRP), a non-specific inflammatory marker, is mainly expressed by the liver and has been proposed as a non-invasive marker of NASH,
- Inflammation regulates hepcidin expression, an important actor in iron homeostasis.
- Osteopontin, a Th1 cytokine and chemokine, plays an important role in the development of insulin resistance and liver complications in dietary murine models.

Findings

- Human adipose tissue expresses CRP, Hepcidin and Osteopontin, and their expression is induced by inflammation;
- Serum CRP levels are not predictive of NASH but are associated with obesity and liver steatosis;
- As a consequence of hepatic and adipose tissue hepcidin expression, low grade inflammation associated with obesity could prevent the hypersideremia-induced liver complications;
- Elevated expression of osteopontin is related to adipose tissue macrophage accumulation and liver steatosis in morbid obesity;

- The liver of obese patients without any histological abnormalities already displays a low-grade inflammation and could be sensitized to activators of the TLR pathway;
- Hepatic inflammation is associated with the regulation of specific genes encoding chemokines and chemokine receptors involved in leukocyte recruitment, CD and cytokines involved in the T cell activation towards a Th1 phenotype, and immune semaphorins;
- A simple and non invasive scoring system using ALT, Metabolic Syndrome and K18 fragments (marker of hepatocyte apoptosis) is able to accurately predict NASH in morbid obesity
- **Emerging concept:** The pathophysiological mechanisms of the progression of steatosis to steatohepatitis are complex and multifactorial. These factors are both from hepatic and extra-hepatic origin, particularly from the adipose tissue. In order to acquire more insight into the pathogenesis of human NAFLD, future studies should focuss on (1) hepatic and adipose tissue of osteopontin signalling, (2) hepatic chemokines and immune semaphorins and (3) inflammation–induced hepatocyte death in the progression of liver complications.

References

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Metabolic profiling in progression of non-alcoholic fatty liver disease

Background

- Current NAFLD diagnosis methods (e.g., liver biopsy analysis or imaging techniques) are poorly suited as tests for such a prevalent condition, from both a clinical and financial point of view.
- This work aims to demonstrate the potential utility of serum metabolic profiling in defining phenotypic biomarkers that could be useful in NAFLD management.
- A parallel animal model/human NAFLD exploratory metabolomics approach was employed, using ultra performance liquid chromatography-mass spectrometry (UPLC-MS) to analyze serum samples collected from non-diabetic, morbidly obese, biopsy-proven NAFLD patients, and animals belonging to the glycine N-methyltransferase knockout (GNMT-KO) NAFLD mouse model.

Findings

- Many of the altered metabolites observed could be associated with biochemical perturbations associated with liver dysfunction (e.g. reduced Creatine) and inflammation (e.g. eicosanoid signaling).
- UPLC[®]/MS metabolic profiling was found to be a suitable platform for the study of NAFLD.
- The metabolite profiles obtained revealed NAFLD perturbations that may be further exploited for future research in disease pathogenesis and development, or harnessed for use in diagnosis, monitoring, and treatment development applications.

Reference

- Barr J ... & Mato JM. Liquid chromatography-mass spectrometry-based parallel metabolic profiling of human and mouse model serum reveals putative biomarkers associated with the progression of nonalcoholic fatty liver disease. *J Proteome Res* 2010;9:4501-12

Non-alcoholic fatty liver disease induces portal hypertension

Background

- In the methionine-choline deficient diet-induced rat model of steatosis, it was shown that steatosis, in the absence of fibrosis and morphological signs of inflammation, induces significant portal hypertension (PHT), associated with systemic vascular hyporeactivity, increased splanchnic blood flow and signs of a hyperdynamic circulation.
- The relevance of these findings for human pathology is unknown to date.

Findings

- We are able to show for the first time in humans that NAFLD can be associated with PHT in the absence of significant fibrosis.
- PHT correlates with the degree of steatosis, which seems to be an independent predictor of the presence of NAFLD-associated PHT.
- The steatosis-associated PHT is an indicator of important changes in liver haemodynamics that might contribute to different types of steatosis-related liver disease.

Reference

- Francque S ... & Michielsen P. Noncirrhotic human nonalcoholic fatty liver disease induces portal hypertension in relation to the histological degree of steatosis. *Eur J Gastroenterol Hepatol* 2010;22:1449-57

Visceral adiposity and insulin resistance predicts NAFLD-related portal hypertension

Background

- In the methionine-choline deficient diet-induced rat model of steatosis, it was shown that steatosis, in the absence of fibrosis and morphological signs of inflammation, induces significant portal hypertension (PHT), associated with signs of a hyperdynamic circulation.
- The relevance of these findings for human pathology was unknown to date.
- We could show that also in patients, NAFLD can be accompanied by PHT regardless of the degree of fibrosis. The degree of steatosis is the most important histological determinant of this PHT.

Findings

- Waist circumference, waist-hip ratio and visceral fat measured by computed tomography are significantly different between patients without and with PHT.
- Fasting insulin, fasting c-peptide and HOMA-IR are significantly different between patients with and without steatosis-induced PHT.
- We are able to identify parameters of visceral fat accumulation and of insulin resistance as independent predictors of the presence of PHT associated with NAFLD.
- Liver fat, visceral fat and insulin resistance and their complex interplay are important in the pathogenesis of steatosis-related liver disease.

Reference

- Francque S ... & Van Gaal L. Visceral adiposity and insulin resistance are independent predictors of the presence of non-cirrhotic NAFLD-related portal hypertension. *Int J Obes* 2010;35:270-8

Farnesoid X receptor induces fetuin-B gene expression in hepatocytes

Background

- FXR regulates bile acid, lipid and glucose metabolism and was suggested to be implicated in the development of certain cancers.

- Fetuin-B was reported to be overexpressed in certain cancers.

Findings

- Fetuin-B is a new FXR target gene in human hepatocytes.
- FXR induces the expression of the fetuin-B gene in human hepatocytes (primary cells and cell line HepG2), but not in murine hepatocytes (primary).

Reference

- Murakami T ... & Staels B. The farnesoid X receptor induces fetuin-B gene expression in human hepatocytes. *Biochem J* 2007;407:461-9

Progression of NASH to hepatocellular carcinoma

Background

- Several molecular mechanisms involving the role of p53 in the progression of NASH to HCC have been identified.
- It has been previously described that kinases LKB1, AMPK and the RNA binding protein HuR are important mediators in a normal liver proliferation.

Findings

- LKB1 has been identified as a hallmark in HCC progression from NAFLD disease. LKB1 is highly activated in HCC derived from NASH. LKB1 controls Akt activation in a PI3K, AMPK and mTOR independent manner. In addition, LKB1 retains p53 in the cytoplasm inhibiting the antiapoptotic features of this protein and avoiding the degradation for its mayor regulator, Mdm2.
- Finally, HuR plays an important role in HCC progression from NASH disease stabilizing factors implicated in angiogenesis, cytokines and oncogenes. HuR levels are highly abundant in HCC derived from NASH.

References

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- Vázquez-Chantada M ... & Martínez-Chantar ML. HuR/methyl-HuR and AUF1 regulate the MAT expressed during liver proliferation, differentiation, and carcinogenesis. *Gastroenterology* 2010;138:1943-53

Coordinators epilogue

Changes in waist circumference and mortality

Background

- Obesity and weight gain increases the risk of premature mortality, but most long-term population-based studies have shown that weight loss is associated with increased mortality
- Individuals differ in their regional distribution of body fat, which have implications for their morbidity and mortality.
- Anthropometric measures of abdominal fatness (e.g. waist circumference (WC)) appear to be more strongly associated with mortality than anthropometric measures of general fatness (e.g. body mass index (BMI))
- The association between mortality and changes in the localization of body fat is not clear.

Findings

- This prospective study of healthy middle-aged men and women showed that changes in WC were positively associated with mortality, whereas changes in BMI were inversely associated with mortality.
- These findings suggest that beneficial effects of weight loss depend of abdominal fat loss
- Development of prevention and treatment strategies targeted against redistribution of fat mass towards the abdominal region is needed.

Reference

- Berentzen TL ... & Sørensen TIA. Changes in waist circumference and mortality in middle-aged men and women. PLoS One 2010;5:9.

Do we need a paradigm shift in the definition of obesity?

Background

- Triacylglycerides (TAG) appears to be biologically inert, primarily serving as a safe energy reserve, whether stored in adipose tissue or ectopically, for example in the liver and skeletal muscles.
- HEPADIP has shown that although there is a correlation between TAG accumulation, especially in the abdominal area and in the liver, and insulin resistance, inflammatory processes, tissue damage, fibrosis formation, these problems are worse when TAG accumulation is limited and less when TAG accumulation is facilitated.
- A variant in the adiponutrin gene (*PNPLA3*) is associated with TAG accumulation in the liver, but less metabolic alterations (insulin resistance, disturbed glucose homeostasis, dyslipidaemia).
- Obesity has been defined on the basis of the association between amount of TAG accumulated in the body and the associated increased health risks.
- The implications of the inert TAG but limitations of its storage need to be unfolded.

Findings

- The amount of accumulated TAG may be considered a confounder of the underlying pathological process
- Measurement of amount of accumulated TAG in the body, whether in adipose tissue or ectopically, in relation to health risks makes sense only if the capacity of TAG accumulation can also be assessed, the relevant question being how close the individuals are at the limits.
- The biology and determinants of the limits of TAG accumulation need to be investigated.
- The understanding of the determinants – whether genetic, environmental or both - of the process of the minute TAG accumulation ending up in obesity and its relation to energy imbalance needs to be interpreted in the frame of the limits of TAG accumulation.
- The biology and determinants of the overloading of the capacity for TAG accumulation need to be investigated.
- The damaging effects of the TAG overloading need to be further investigated along the lines of HEPADIP
- Preventive and therapeutic strategies should aim at keeping the TAG accumulation below its limits.
- The obesity epidemic needs to be reinterpreted; in view of the epidemic of diabetes and expectedly ensuing cardiovascular diseases in the Asian populations in which the capacity for TAG accumulation appears much lower than in the Western populations, the obesity epidemic may have been partially saved from a similar increase in diabetes at an earlier stage.

References

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