Abstract book

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Mast cell deficient mice are protected from diet-induced obesity, and have altered immune cell infiltration in their adipose tissue independent of high fat diet

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Introduction: Obesity is associated with adipose tissue (AT) inflammation and dysfunction. It has previously been shown that mast cells play a role in the development of obesity and type 2 diabetes. Mast cell deficient mice gain less weight and are more insulin sensitive after a high fat diet (HFD). In this study we aimed to further characterize the effects of mast cell deficiency on adipocyte function, as well as the inflammatory status of AT and the circulation.

Methods: Characterization of both mast cell deficient Sash (*W-sash* c-*kit* Mutant *Kit*$^{W\text{-}sh/W\text{-}sh}$ Mice) and WT mice (both n=10) was performed after 8 weeks on chow and on HFD (45% lard). Body weight and adipocyte morphology and function were analyzed. B-cell, T-cell, and macrophage/monocyte numbers were determined in visceral and gonadal AT and in the circulation by flow cytometry.

Results: Sash mice gained significantly less body weight on HFD compared to WT mice, which was associated with smaller fat pads containing smaller adipocytes. Compared to chow, HFD did not induce weight gain in Sash mice. Adipocytes from Sash and WT mice showed similar 8-Bromo-cAMP induced lipolysis and insulin inhibition thereof. Sash mice, both on chow and HFD, had significantly increased numbers of T-cells in their AT and circulation, and reduced macrophage infiltration in their AT.

Conclusion: Mast cell deficient mice are protected from diet-induced obesity, with altered macrophage and T-cell infiltration in AT, independent of HFD. These data suggest that mast cells play a regulatory role in leukocyte infiltration and WAT expansion.

First evidence of brown adipose tissue recruitment in humans after cold acclimation

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Background: Mild cold exposure increases energy expenditure without shivering, called non-shivering thermogenesis (NST). In rodents, brown adipose tissue (BAT) is the major contributor to NST. Nowadays, it is well recognized that active BAT depots are present in human adults. This study aimed to elucidate the role of BAT, skeletal muscle and white adipose tissue in human adults, during cold stimulation.

Methods: 17 young and lean humans participated (9F/8M). Cold acclimation was achieved by exposure to an ambient temperature of 15-16 °C for 6 hours a day during 10 consecutive days. Before and after this period an abdominal subcutaneous fat biopsy and muscle biopsy were taken. Cold-induced BAT activity was measured using [^{18}F]FDG-PET/CT-imaging during maximal NST, with simultaneous energy expenditure (indirect calorimetry) measurements. VAS-scales on sensation, comfort and shivering were completed during cold acclimation.

Results: Cold acclimation increased both BAT activity (2.4±0.7 to 2.8±0.5 SUVmean ($p<0.01$)) and detectable BAT volume (665±451 to 913±458 cc ($p<0.01$)). In parallel an increase in NST was found (10.8±7.5 to 17.8±11.1 % ($p<0.01$)). Cellular respiration measurements revealed no significant changes in skeletal muscle mitochondrial uncoupling upon cold acclimation. No effect of cold acclimation on factors related to 'browning' of abdominal subcutaneous white adipose tissue was found. After cold acclimation, subjects judged the environment warmer ($p<0.05$), felt more comfortable ($p<0.01$) and reported less shivering ($p<0.01$) during cold exposure.

Conclusion: A variable indoor environment with frequent cold exposures might contribute to counteract the current obesity pandemic, this might be an acceptable and economic manner to increase energy expenditure.

High dietary protein intake results in lower intra hepatic lipid content in healthy humans on a hypercaloric high-fat diet

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Background: High-protein diets may improve regulation of body weight and glucose homeostasis and, at least in animals, reduce intra hepatic lipid content. Our objective was to reduce intra hepatic lipids (IHL) in healthy humans, by increasing protein (P) intake at the expense of carbohydrates (CH), in a high-fat-hypercaloric-diet.

Methods: A strictly controlled dietary intervention was performed in lean healthy subjects, who were randomly assigned to the intervention groups. One group received a control-diet (CD-group; n=10) (28 en% fat; 17 en% P; 55 en% CH) for 4 weeks. The other group (n=17) received a hypercaloric high-fat diet (HD-group; + 2 MJ per day, 40 en% fat) in a 2-week cross-over design, with a high protein content (HP; 26 en% P) or a normal protein content (NP; 15 en% P) and vice versa.

Results: On the HD diet there was a trend for lower IHL on HP compared to NP (difference 0.2 %, p=0.08); and a trend for increased IHL after BD compared to HD was seen (difference 0.13 %,p=0.06).
Body weight did not differ between HP and NP, however fat mass was lower (-0.27 kg, p=0.02) and lean mass was higher (+0.52 kg, p=0.003) on HP compared to NP. Plasma triglyceride levels tended to be lower on the HP vs. NP (p=0.09). Fasting insulin sensitivity did not differ between protein diets.

Conclusion: High dietary protein intake, at the expense of carbohydrates, resulted in lower IHL in healthy humans, receiving a hypercaloric high-fat diet.

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Visceral Fat more Important than Hepatic Fat in the Etiology of Insulin Resistance: the NEO Study

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Background: Fatty liver is associated with type 2 diabetes. However, it is unclear whether hepatic triglyceride accumulation itself causes diabetes, or merely is a consequence of excess adipose tissue, that is related to insulin resistance. We aimed to investigate the association between hepatic triglyceride content (HTGC) and insulin resistance, and whether this association could be explained by measures of adiposity.

Methods: In this cross-sectional analysis of the Netherlands Epidemiology of Obesity (NEO) study, fasting glucose and insulin concentrations were measured. HTGC was measured using magnetic resonance spectroscopy, visceral adipose tissue (VAT) with MRI, total body fat (TBF) with bioelectrical impedance analysis. We performed linear regression analysis of HTGC with the updated homeostasis model assessment (HOMA2-IR), adjusting for age, sex, ethnicity, education, smoking, alcohol consumption, VAT and TBF.

Results: After exclusion of participants with missing data (n=65), diabetes (n=69), alcohol consumption>4 glasses/day (n=72), 1,160 participants were included with a mean (SD) age: 55 (6) years, mean fasting glucose: 5.6 (0.8) mmol/L, 52% men. One SD HTGC (9.6%) was associated with an increase in HOMA2-IR of 37% (95%-CI:22%,54%), this attenuated to 23% (95%-CI:11%,36%) after adjustment for VAT and to 21% (95%-CI:10%,32%) after additional adjustment for TBF. In this full model, one SD VAT (0.06 dm^3) was associated with an increase in HOMA2-IR of 27% (95%-CI:18%,36%) and one SD TBF (men:5.0%, women:4.7%) with 19% (95%-CI:12%,27%).

Conclusion: The association between hepatic fat and insulin resistance was for a large part explained by VAT. VAT may be most important in the etiology of insulin resistance.

Long-Term Successful Medical Treatment Of Severe Obesity: The GET-REAL Program

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Introduction: Disappointing long-term results from medical treatment have led to the increasing application of bariatric surgery in class 3 obesity (CL3OB). We developed a behavior modification program, aiming to provide an alternative for surgery.

Methods: 79 adults with CL3OB or CL2OB with major complications of obesity (BMI 44.5 ± 0.7 kg/m^2) followed group lessons alternated with individual consultations with highly trained professionals. Individualized exercise and diet instructions (1200-2000 kCal/day) were given. 109 controls (BMI 43.2 ± 0.5 kg/m^2) received standard care.

Results: 2-year weight loss averaged 18.0 ± 1.8 kg* (14 ± 1% of IBW*, 24 ± 2% of excess body weight (EBW)*). 32 patients lost ≥ 10% of IBW, 13 of whom ≥ 20%, and 3 ≥ 30%. Controls gained 0.9 ± 0.7 kg (0.8 ± 0.6 % of IBW, 2.9 ± 1.3% of EBW), 2 people losing ≥ 10% IBW. Intention-to-treat analysis assuming either total relapse or weight maintenance after dropout showed mean losses of 12.7 ± 1.6 kg* (9.6 ± 1.1 % of IBW*, 17.3 ± 2.1% of EBW*) and 15.4 ± 1.7 kg* (11.4 ± 1.1 % of IBW*, 20.8 ± 2.1% of EBW *) respectively. Preliminary results show weight maintenance of successful subjects up to 42 months. 20-35% of these results would have been considered at least satisfactory by various surgical standards.

Conclusion: Non-surgical mid/long term weight loss is achievable in severely obese patients in outpatient settings; the efficacy/safety trade-off in obesity treatment is an important consideration in interpreting these results.
*$p<0.001$

Perceptions of low SES groups from different ethnic origins towards a healthy lifestyle

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Background: Individuals with low socio-economic status (SES) are less likely to be physically active or follow a healthy diet. This study aimed to explore perceptions of low SES adults from different ethnic origins towards a healthy lifestyle in order to tailor lifestyle interventions to their needs.

Methods: In this study, 14 focus group interviews were held with groups of Moroccan, Turkish and Dutch ethnicity with a low SES. Participants were encouraged to share their perceptions towards healthy eating and physical activity.

Results: In all groups uncertainty about the definition of a healthy diet and sufficient physical activity was reported. Furthermore, participants described a lack of energy at certain moments to maintain demanding health behaviours. Moreover, the lack of a supportive environment was mentioned as making lifestyle change difficult. On the other hand, social support was frequently named as important enabler to initiate or maintain health behaviours. However, participants seemed to struggle to combine a healthy lifestyle with other priorities in daily life. They indicated that the absence of stress and moments of pleasure are also relevant factors that contribute to their health.

Conclusions: Our results indicate that health behaviour is deeply embedded in an individual's social context, which can make behaviour change difficult regardless of a person's ethnic origin. They emphasise that advice needs to be tailored to the individual's personal challenges and social context. Increasing social support and creating enjoyable moments in lifestyle interventions are identified as potential enablers for participants in order to initiate and maintain demanding health behaviours.

A Hypercaloric Snacking Diet Increases Liver Fat in Lean Men within 6 Weeks

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Introduction: Hepatic steatosis predisposes to fatty liver disease and is associated with visceral obesity. Also, fat accumulation in the liver reduces hepatic insulin sensitivity which increases plasma glucose concentrations. In addition to ectopic fat accumulation, excessive intake of dietary sugars and fat has been suggested to stimulate triglyceride storage in the liver. In the current study we investigated the role of dietary composition and diet pattern on liver fat accumulation and hepatic insulin sensitivity.

Methods: 29 lean male subjects (age: 22.0±2.5yrs; BMI: 22.3±1.3 kg/m^2) followed a hypercaloric diet with 40% caloric surplus on top of their regular diet for 6 weeks. Subjects were randomised into 1 of 4 diet groups: increasing meal size (S) or meal frequency (F) while on a hypercaloric high sugar (HS) or hypercaloric high fat high sugar (HFHS) diet. Before- and after the diet we measured liver fat with MRspectroscopy (^{1}H-MRS) and hepatic insulin sensitivity with a hyperinsulinemic euglycemic clamp.

Results: Weight significantly increased in all 4 diet groups, yet remained within normal BMI ranges (20-25 kg/m^2). Liver fat increased significantly in the HFHS-F and the HS-F group, but not in the groups with increased meal size. We observed a trend towards a decrease in hepatic insulin sensitivity in the HFHS-F group (p=0.08), but not in the HS group.

Conclusions: 6 weeks of hypercaloric snacking with either fat/sugar or sugar alone increases liver fat, but only snacking fat/sugar tended to decrease hepatic insulin sensitivity. These data suggest that a hypercaloric snacking diet itself can contribute to hepatic steatosis independently of visceral adiposity.

The impact of long-term BMI patterns on quality of life. The Doetinchem Cohort Study

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Background: Overweight is associated with a reduced health-related quality of life (QoL), but less is known about the impact of long-term body mass index (BMI) patterns on QoL in adults.

Methods: In the Dutch Doetinchem Cohort Study (1989–2009), 1677 men and 1731 women aged 20-66, six BMI patterns were defined using four measurements over a 15-year period: persistent healthy weight (18.5-24.9 kg/m^2, reference pattern), persistent overweight (25.0-29.9 kg/m^2), persistent obesity (≥30.0 kg/m^2), developing overweight, developing obesity and switching between BMI categories. Eight dimensions of QoL were assessed with the SF-36 questionnaire on a 0-100 scale. Multivariable-adjusted QoL was estimated at the end of the 15-year period.

Results: The lowest QoL was observed for persistent obesity of all BMI patterns. It was 5.0 points ($p=0.02$) lower for one mental dimension in men and 6.2-11.6 points ($p<0.05$) lower for five (mainly physical) dimensions in women. Developing overweight or obesity scored 1.8-6.3 points ($p<0.05$) lower on 2-5 (mainly physical) dimensions. Persistent overweight did hardly differ from a persistent healthy weight. In women, switching between BMI categories resulted in a lower QoL on the mental dimensions.

Conclusions: Studying long-term BMI patterns over a 15-year period showed that persistent obesity, developing overweight and developing obesity resulted in a lower QoL –particularly on the physical dimensions– compared to a persistent healthy weight. In particular for women, prevention of developing overweight and especially obesity is not only important for preventing specific diseases, but also for QoL in general.

Brown adipose tissue and thyroid hormone in humans

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Background: The last few years studies on human brown adipose tissue (BAT), in relation to prevention and treatment of obesity has significantly increased. Thyroid hormones have a positive effect on BAT activity by directly (systemic) influencing on BAT sensitivity to the sympathetic nervous system and by stimulation of the hypothalamus, thereby activating the sympathetic pathway. The primary objective is to study the effect of thyroid hormone on brown adipose tissue activity. Secondary endpoints are the effect of thyroid hormone on energy metabolism, body core temperature, skin surface temperature and skin perfusion.

Methods: BAT presence and activity is analyzed in patients during the two different phases of the treatment of well-differentiated thyroid cancer with a total thyroidectomy and subsequent radioactive iodine ablation. The first treatment phase follows total thyroidectomy, in which the thyroid hormone levels gradually become low or non-existent. The second phase is after radioactive iodine ablation, in which TSH-suppressive doses of thyroid hormone are administered to the patients until a euthyroid near-to-hyperthyroid hormone level status is reached. ^{18}F-FDG PET-CT-imaging will be performed under mild cold stimulation to assess the BAT presence and activity in the different treatment phases.

Results: Preliminary results of the study show a positive effect of thyroid hormone suppletion on BAT presence and activity in patients compared to BAT activity in a hypothyroid hormone status.

Conclusion: The relation between thyroid hormones and brown adipose tissue activity has not been proved in humans yet. In this study we hypothesize that thyroid hormone levels are related to BAT activity in man.

Postprandial blood pressure and hormone responses differ after intake of different proteins

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Background: Hypertension is a comorbidity often seen in obesity. It has been shown that consumption of a protein mix compared to maltodextrin lowers blood pressure (BP) (1). Some hormones may have vasoactive properties, like insulin, glucagon like peptide 1 (GLP-1) and glucagon. The aim of this study was to compare postprandial changes in BP and these hormones between three different protein sources.

Methods: Pea, milk and egg protein isolate, were tested in a randomized order by a crossover design with one week washout. Postprandial responses of BP, plasma glucose, insulin, glucagon and GLP-1 were monitored for four hours in men and women (BMI 25-35 kg/m^2) with untreated elevated BP.

Results: Postprandial mean arterial pressure was higher after egg protein consumption compared to milk and pea protein from 2-4 hours ($P \leq 0.008$). Glucose responses did not differ between proteins. Compared to the other proteins, egg protein induced lower insulin concentrations from 1-2 hours and lower GLP-1 at 2 hours ($P < 0.0001$). Plasma glucagon differed between all three protein sources from 1-2 hours with pea>milk>egg ($P \leq 0.003$).

Conclusion: This study shows that different proteins can induce different postprandial responses in BP, insulin, GLP-1 and glucagon. Dietary proteins may modulate BP via differences in hormonal responses. Whether prolonged consumption of these proteins will also lead to long-term differences in BP remains to be determined.

References

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Apolipoprotein A5 deficiency aggravates high fat diet induced obesity due to impaired regulation of satiety signalling

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Background: Mutations in apolipoprotein A5 (*Apoa5*) have been associated with hypertriglyceridemia in humans and mice. This has been attributed to a stimulating role for APOA5 in lipoprotein lipase-mediated triglyceride hydrolysis and hepatic clearance of lipoprotein remnant particles. However, due to the low APOA5 plasma abundance, we investigated an additional signaling role for APOA5 in high fat diet (HFD) induced obesity.

Methods: Wildtype and *Apoa5*$^{-/-}$ mice were put on chow and HFD and food intake (FI) was recorded. FI was also measured in wildtype mice intravenously injected with human APOA5-loaded VLDL-like particles and in *Apoa5*$^{-/-}$ mice injected with adenovirus mediated hepatic overexpression of *APOA5.* Finally, after intracerebroventricular injection of artificial cerebral spinal fluid / APOA5 / Neuropeptide Y in wildtype mice, FI was measured.

Results: Wildtype and *Apoa5*$^{-/-}$ mice on chow diet showed no difference in bodyweight or 24h FI, while on HFD *Apoa5*$^{-/-}$ mice ate more in 24h (*Apoa5*$^{-/-}$, 2.8±0.4g, WT, 2.5±0.3g, $p<0.05$) and became more obese then wildtype mice. Also, intravenous injection of APOA5-loaded VLDL-like particles lowered FI (VLDL-control, 0.26±0.04g, VLDL+APOA5, 0.11±0.07g, $p<0.01$). In addition, the HFD induced hyperphagia of *Apoa5*$^{-/-}$ mice was prevented by adenovirus mediated hepatic overexpression of *APOA5*. Finally, intracerebroventricular injection of APOA5 reduced FI compared to injection of the same mouse with aCFS (aCSF, 0.40±0.11g, APOA5, 0.23±0.08g, $p<0.01$).

Conclusion: These data indicate that the increased HFD-induced obesity of *Apoa5*$^{-/-}$ mice as compared to WT mice is at least partly explained by hyperphagia and that APOA5 plays a role in the central regulation of food intake.