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Scientific coordination of the German-French Diabetes Research Academy:
Christian Boitard, Hans-Ulrich Häring, Martin Hrabě de Angelis, Emmanuel Van Obberghen

Abstracts of Posters

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A Nicotinamide Riboside based strategy for the treatment of binge alcohol-induced liver damage

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Binge drinking has become an alarming health problem associated with aggravated liver injury. In the liver, alcohol oxidation results in massive depletion of cellular nicotinamide adenine dinucleotide (NAD^+), a key cause for alcohol induced liver complications due to the inhibition of NAD^+ dependent deacetylases sirtuin (SIRT) 1 and 3 in the cytosol and the mitochondria, respectively. These two enzymes are crucial regulators of liver lipid metabolism, mitochondrial biogenesis, oxidative stress and inflammation via the deacetylation of several molecular targets including histones, transcription activators and co-activators. We aim to determine whether an acute oral supplementation of nicotinamide riboside (NR), an NAD^+ biosynthesis precursor, protects against alcohol induced liver damage in a mouse model of binge drinking. We show that an acute single oral dose of NR attenuates binge alcohol induced hepatic steatosis, circulating transaminases, and oxidative stress in the liver. NR treatment also helps to better assimilate alcohol and decreases accumulation of the toxic alcohol metabolite acetaldehyde. These benefits seam to rely on the reversal of alcohol induced protein hyperacetylation specifically in the mitochondria. In addition, we confirm the potential of NR treatment in reversing alcohol induced liver damage in a chronic binge drinking protocol. In the face of the mounting prevalence of binge drinking and the lack of efficient therapies against alcoholic liver disease, this study opens exciting perspectives for the treatment of multiples facades of alcohol induces liver damage.

Metabolic sensing and transcriptional activity of Irf5 functionally redefines metabolic adaptation of innate immune cells in diabetes

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Interferon regulatory factor (Irf)-5 mediates macrophage pro-inflammatory differentiation; physiologically responding to TLR-ligands and pathologically linked to autoimmune disorders. We recently described novel pathogenic roles of Irf5 in adipose tissue and liver macrophages in diabetes (Dalmas et al 2015, NatMed; Alzaid et al 2016 JCI Insight). Despite these advances, the specific stressors inducing Irf5-dependent inflammation, and their intracellular handling in diabetes, remain unknown. The current study characterises Irf5's dynamic regulation by candidate metabolic stressors and the cellular adaptation mediated by Irf5's activation. We present a series of human studies, *in-vivo* and *in-vitro* models mechanistically evaluating the immunogenicity of dysmetabolism. We report that specific lipotoxic factors stimulate Irf5, independently of other stressors. Lipotoxic signalling induces Irf5-dependent inflammation and alters cellular metabolic flux, a process necessary for effective inflammation. Interestingly, we found that Irf5 is induced, not only in macrophages, but also in their circulating monocyte progenitors early in disease modelling. Analyses of the Irf5-cistrome, transcriptome and intracellular metabolome led to the identification of novel target enzymes and metabolic intermediates controlled by Irf5 and instrumental to metabolic adaptation of macrophages. Importantly we highlight for the first time that Irf5 plays an unexpected role in adapting cellular metabolism under diabetic dysmetabolism, altering metabolic adaptation and in-turn inflammation. We report unanticipated roles of Irf5 acting as a relay and adaptor for dysmetabolism, these findings highlight novel therapeutic targets and a potential predictive biomarker in Irf5 from circulating cells.

Dietary regulation of Facilitative Glucose Transporters GLUT3 and GLUT5 in abdominal subcutaneous adipose tissue of type 2 diabetes patients

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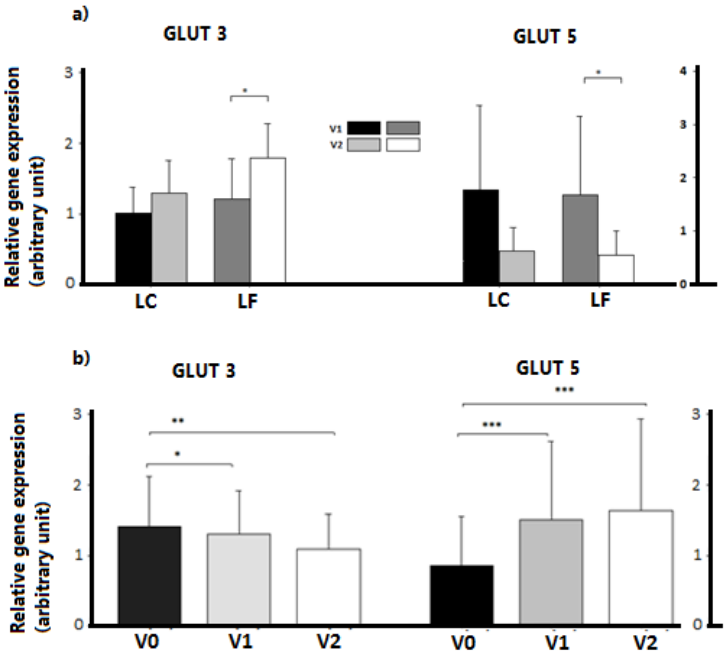
Background: In health, GLUT transporters have different expression patterns depending on the tissue and their abundance is regulated according to the metabolic need. However, an unusual expression of GLUT protein was observed in several diseases including type 2 diabetes mellitus (T2DM) and adiposity. Evidence suggests that diet may be essential in regulating GLUT gene expression.

Figure 1: Results



Values are presented as means (±SD). The statistical significance level was set at **<0.05.** *P<0.05; **P<0.01; ***P<0.001.
a) LC vs LF: paired t-test for difference within diet groups, independent t-test for difference between diet groups.
b) HF 1 week vs 6 weeks: ANOVA, repeated measurements.

Objective: The primary objective of this study was to investigate the short term effects of a Low-Fat (LF) diet (<30% Fat, 50% Carbohydrate, 20% Protein, 1000-1200kcal/d) and a Low-Carb (LC) diet (60-70% Fat, 5-10% Carbohydrate, 20-30% Protein, 1200-1500kcal/d) focusing on diet induced regulation of GLUT transporters in abdominal subcutaneous adipose tissue (SCAT) of T2DM patients. The secondary objective was to compare GLUT transporters regulation of T2DM patients under previously described diets with the regulation of healthy, non-adipose subjects, which underwent 6 weeks on a high fat, isocaloric (HF) diet (45% fat, 40% carbohydrate, 15% protein) after 6 weeks standardization (LF) diet (30% fat, 55% carbohydrate, 15% protein).

Methods: Seventeen patients were randomly assigned either to the LC diet or to the LF diet. SCAT biopsies were obtained at baseline and after the 3 weeks dietary intervention. In addition, SCAT of 92 healthy, non-obese adults, who went through a HF diet, was provided after 1 and 6 weeks. RT-PCR was employed to quantify the expression of GLUT transporters.

Results: As shown in Figure 1a after the LF diet GLUT3 mRNA levels increased significantly and GLUT5 mRNA levels showed a significant reduction. On the contrary, after a HF diet (Figure 1b) GLUT3 mRNA levels decreased significantly, while GLUT5 expression showed a significant up-regulation.

Conclusion: Low fat, hypocaloric diet may significantly up-regulate GLUT3 expression and down-regulate GLUT5 expression in SCAT of T2DM patients and controls. The significance is unclear but may relate to the differentiation of adipose tissue.

Identification and characterization of rare mutations in *MRAP2*: towards a new mechanism linking obesity and diabetes

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Type 2 diabetes (T2D) results from the progressive dysfunction of insulin secretion in pancreatic β cells, in the background of insulin resistance. Obesity is one of the major risk factors for T2D development. Our lab and others found that rare mutations in ~50 genes cause monogenic forms of diabetes or obesity. Recently, a study showed that the absence of melanocortin 2 receptor accessory protein 2 (MRAP2) is associated with mammalian obesity, and that rare *MRAP2* mutations could cause monogenic obesity in humans. MRAP2 is a G protein-coupled receptor (GPCR) accessory protein that regulates the signaling of melanocortin receptors (MCRs). The role of MRAP2 in energy homeostasis and obesity could be explained by its interactions with MC4R in the hypothalamus. Here, we aimed to identify rare mutations of *MRAP2* in a large-scale resequencing project, to analyze their putative association with obesity, and to investigate the functional effect of each MRAP2 mutant in cell models. We found that rare *MRAP2* mutations are associated with familial severe obesity but also with familial T2D. We showed that MRAP2 is expressed in human pancreatic islets and β cells (in addition to hypothalamus), suggesting a role in T2D development. Among MCRs, only MC1R is expressed in pancreatic islets and β cells. We are now investigating by luciferase assays in CHO cells the functional interaction of each *MRAP2* mutants with MC1R and MC4R in response to the ligands ACTH and αMSH. Then, we will analyze the effect of deleterious *MRAP2* variants only on obesity and/or T2D risk.

SIRT3 deficiency exacerbates high-fat diet-induced hepatic steatosis: Potential involvement of CD36 and VLDLR

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Non-alcoholic fatty liver disease is an important risk factor for the development of obesity-related pathologies including insulin resistance and type 2 diabetes mellitus. SIRT3 is a member of the sirtuin family of protein deacetylases that resides primarily in the mitochondria, binding and deacetylating several metabolic enzymes that regulate important mitochondrial functions. One key role of SIRT3 is to regulate fatty acid metabolism. In fact, exposure to a high-fat diet (HFD) exacerbates obesity, insulin resistance, dyslipidemia, fatty liver, and hepatic inflammation in SIRT3-deficient mice compared with wild-type (WT) mice. In this study, we aimed to explore new mechanisms by which SIRT3 deficiency can exacerbate hepatic steatosis. WT and $SIRT3^{-/-}$ mice were fed a HFD (55% kcal from fat) for a period of 6 months. HFD supplementation exacerbated hepatic triglyceride content in $SIRT3^{-/-}$ mice compared with WT mice fed the HFD. In addition, WT mice fed a HFD showed an increase in the expression of PPARα-target genes, but this increase was prevented in the liver of Sirt3-deficient mice fed a HFD. These changes were accompanied by an increase in HIF-1α and Lipin1 in Sirt3-deficient mice that was prevented when these mice were fed a HFD. Next, we examined the levels of two proteins involved in lipid transport, CD36 and VLDL-R, which are under the control of Nrf2 and PPARγ. A significant increase was observed in the protein levels of CD36 and VLDL-R in SIRT3-deficient mice fed a HFD compared with the same mice fed a standard diet. Consistent with this, the protein levels of NQO1, a Nrf2-target gene, and of PPARγ were increased in SIRT3-deficient mice fed a HFD compared with the same mice fed a standard diet. Overall, these findings suggest that feeding a HFD in the presence of SIRT3 deficiency exacerbates hepatic steatosis by increasing CD36 and VLDL-R levels.

Role of co-inhibitory molecules in pancreatic **β** cell/CD8^{+} T lymphocyte interaction.

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Type 1 diabetes results from a failure in central and peripheral tolerance, leading to progressive destruction of insulin-producing pancreatic β cells, mediated by autoreactive T lymphocytes. Co-inhibitory molecules play a critical role in the regulation of T cell activation and in the maintenance of immune homeostasis. Indeed, several co-inhibitory receptors (PD1, TIGIT, LAG3) have been genetically associated with susceptibility to multiple autoimmune diseases.
The aim of this study is to investigate the role of co-inhibitory molecules during pancreatic β cell and CD8+ T lymphocytes interaction. We analyzed the basal expression of co-inhibitory receptors in autoreactive CD8+ T lymphocyte clones, and of their ligands in control and cytokine stimulated human β cell line (ECN90). Using an in vitro assay modeling β cell destruction by CD8+ T cell clones (Culina et al., Sci Immunol., 2018) we analyzed the kinetic of expression of these co-inhibitory receptors and ligands on CD8+ T-cells and β cell line at early and late stages of the T cell response. We will further investigate their potential protective role by modulating their expression on β cells and CD8+ T cells, or blocking their interaction.

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Superficial Subcutaneous Adipose Tissue Thickness, Mitochondrial Efficiency and Stearic-to-Palmitic Acid Ratio are Decreased in Humans with Type 2 Diabetes

Whole abdominal subcutaneous adipose tissue (WSAT) is divided into deep (DSAT) and superficial (SSAT) layers that may have different metabolic properties. In glucose-tolerant humans (CON), insulin sensitivity (IS) correlates positively with markers of lipogenesis such as stearic-to-palmitic acid ratio (18:0/16:0) in SAT and negatively with abdominal DSAT/WSAT thickness. IS further correlates with muscle mitochondrial function. However, SSAT/WSAT thickness, 18:0/16:0 and mitochondrial efficiency remains unknown in SSAT of patients with type 2 diabetes (T2D). We hypothesized that SSAT/WSAT, 18:0/16:0 and mitochondrial efficiency in SSAT are decreased in male patients with T2D compared to CON.

In 14 T2D and 14 CON matched for body mass index (BMI), age and WSAT thickness (32±1 kg/m2, 53±2 yrs, 29±2 mm vs. 31±1 kg/m2, 53±2 yrs, 27±3 mm), we assessed M-values by euglycemic-hyperinsulinemic clamps, SSAT and DSAT thickness by ultrasound imaging. In biopsies of SSAT and DSAT 18:0/16:0 was assessed by gas chromatography-mass spectrometry and maximal mitochondrial oxidative capacity and efficiency, i.e. respiratory control ratio (RCR=state 3/state 4), by high-resolution respirometry.

T2D had 41% lower M-value and 33% lower SSAT/WSAT than CON (both $p<0.001$). In SSAT T2D had lower 18:0/16:0 than CON (0.15±0.01 vs. 0.18±0.01, $p<0.05$). Comparing both compartments 18:0/16:0 was lower in SSAT than in DSAT of T2D (0.15±0.01 vs. 0.17±0.01, $p<0.01$). While maximal oxidative capacity was comparable in both layers, maximal oxidative capacity of SSAT correlated positively with M-value ($r=0.80$, $p<0.001$) in CON upon adjustment for age and BMI. RCR was 33% lower in SSAT of T2D compared to SSAT of CON ($p<0.001$). SSAT/WSAT and 18:0/16:0 did not relate to M-value.

In conclusion, SSAT represents a distinct abdominal SAT compartment with reduced thickness, mitochondrial efficiency and stearic-to-palmitic acid ratio in T2D.

Cigularova, Maya / Fadler, Janine / Kabisch, Stefan / Dambeck, Ulrike / Gerbracht, Christiana / Sachno, Anna / Honsek, Caroline / Pfeiffer, Andreas F.H.

Effects of low-carb and low-fat dietary strategies on renal function in subjects with prediabetes – DiNA-P

Background: Diabetic nephropathy is a severe chronic complication, occuring in up to 30 % of diabetes patients. In prediabetes, a lower incidence of renal dysfunction is reported, but nephropathy can already be present before manifestation of overt diabetes. Both hyperglycemia and renal dysfunction can initially be treated with dietary approaches. Besides mere weight reduction, specific changes in diet composition are usually necessary. Low-fat diets, but possibly even more low-carb diets seem to improve anthropometric and metabolic parameters. As low-carb diets are usually high-protein diets, the safety of this particular aspect needs to be evaluated.

Aims: We compare the effect of a two-phase one-year low-carb and low-fat diet in subjects with prediabetes, focusing on renal function, assessing phase-wise changes and investigate mechanisms of improvement or impairment by correlation analysis.

Methods: Our analysis is conducted within the first and second phase of the ongoing DiNA-D study, covering 12 months of dietary intervention in 150 subjects, comparing both diet groups. Renal function is assessed by creatinine and urea levels, estimated glomerular filtration rate (CKD-EPI equation) and spontaneous albuminuria. Statistical analysis is based on within-group (Wilcoxon tests) and between-group (Mann-Whitney-U-tests) comparisons as well as non-parametric correlation analysis (Spearman). Level of significance is $p<0,05$.

Results: 150 subjects (71 % women), aged 60±9, mean BMI 31,7±5,7 kg/m², with high-risk prediabetes are analysed.
At baseline, the average of all renal parameters was within their specific ranges of reference, but a minor portion of all subjects showed microalbuminuria (>30 mg/L). Within the first diet phase, anthropometric parameters (BMI, blood pressure) improved significantly stronger in the low-carb group. In the second diet phase, no such superiority can be demonstrated.
Within the first diet and second dietary phase, renal parameters did not change with statistical significance, neither within, nor in comparison between both groups. Urea levels initially increased in the low-carb group, mirroring the effect of increased protein intake and urea production but no impairment of renal function. Diet-specific correlation analysis does not consistently reveal modulating factors of renal function.

Conclusions: Within our study, low-fat or protein-rich low-carb diet did not impair renal function in patients with prediabetes. Our preliminary data show, that distinct hypocaloric diets may preserve normal renal parameters. We are looking forward to the completion of recruitment in this year, as more data is required to evaluate dietary effects on prediabetes subtypes.

Ulrike Dambeck; Stefan Kabisch; Caroline Honsek; Christiana Gerbracht; Valeria Filatow; Andrea Böhm; Laura Schulzik; Naghme Mazandarany; Deborah Frisch; Andreas F.H. Pfeiffer

Comparison of low-carb and low-fat diet in prediabetes: Superiority of low-carb after 3 weeks, metabolic equipoise after one year – the DiNA-P study

Background: Hypercaloric dietary patterns are the fuel to T2DM, being linked to all features of the metabolic syndrome and its hepatic manifestation, the nonalcoholic fatty liver disease. Reducing intrahepatic lipids (IHL) may offer an effective therapeutic and preventive strategy
Aims: Within the Diabetes-Nutrition-Algorithm in Prediabetes (DiNA-P)-study we investigate the effect of the sequential hypocaloric short-term, very-low-carb vs. low-fat diet, followed by a longterm isocaloric PUFA-enriched moderately low-carb or low-fat dietary intervention on glycemic parameters, intrahepatic lipids (IHL) and other risk factors for cardiovascular disease in prediabetic subjects.
Methods: DiNA-P includes 250 subjects with high-risk prediabetes and covers one year of dietary intervention, comparing low-carb and low-fat diet in two phases of 3 weeks with intensive initiation diet and 11 months of moderate maintenance diet. Within each diet group, there is an additional randomisation to either 8 or 16 dietary consultations during the intervention. In the long-term period low-carb treatment is accommodated by supplementation with PUFAs (walnuts, muffins) in all subjects. Phenotyping assessment includes anthropometry with neuropathy screening, liver 1H-MRS and whole-body MRI, fasting blood samples and oGTT.
Results: A preliminary cohort of 150 participants was analyzed for both phases. The initial hypocaloric low-carb led to a higher reduction of body weight, fasting glucose, postprandial insulin and C-peptide concentrations (CRP), triglycerides, LDL/HDL ratio and blood pressure. Correlations with body weight change suggest that body weight reduction may not be the major cause for IHL reduction under low-carb, in contrast to LFD. LFD showed higher reductions for LDL-cholesterol and decreased C-reactive protein.
Only on the long-term LFD, subjects maintained their weight reduction as well as their decline in triglycerides and CRP. In the LCD group, fasting glucose, insulin and c-peptide concentration, triglycerides as well as blood pressure re-increased throughout the 11-months treatment. Despite this body weight regain, we observed a significantly stronger reduction of 2-hour oGTT glucose in the low-carb group after one year. Cholesterol increased in all participants, albeit to a higher extent in the PUFA-LCD-group. The latter displayed a considerably decline in CRP, which did not support the suggested proinflammatory properties of n-6-fatty acid-derived metabolites. There was no change in HbA1c, which can be a consequence of the relative low baseline concentrations of prediabetic patients (bottom effect).
The deterioration of insulin sensitivity and insulin resistance respectively may be attributed due to the higher total fat intake, which may have masked a beneficial PUFA-effect.
Conclusions: Our data show, that the two-step diet led to comparable weight reduction and change in body composition in both diet groups. The long-term energy intake seems to be the crucial contributor to maintain IHL-reduction, although a clear statement cannot be drawn given the different caloric intakes in the second study phase. The low-fat concept displayed superiority in the preservation of improved insulin sensitivity and triglyceride levels. Low-carb led to a stronger long-term improvement of the insulin-independent glucose effectivity, possibly compensating the impaired insulin effect, and served beneficial in the long-term improvement of waist circumference, serum lipid and blood pressure profile.

Claudia Diederich, Stefan Kabisch, Ulrike Dambeck, Christiana Gerbracht, Caroline Honsek, Anna Sachno, Andreas F.H. Pfeiffer

Factors predicting dietary compliance in subjects with prediabetes – Data from DiNA-P

Background: Obesity and type 2 diabetes are major challenges in today's public health. A huge variety of dietary RCTs have tried to identify the best way to lose weight and to improve metabolism, the most important strategies being "low-carb" and "low-fat". Recent publications have demonstrated, that low-carb diet may be superior to low-fat with regard to the anthropometric and metabolic outcome. However, dietary compliance seems to be better in low-carb subjects, resulting in slightly higher drop-out rates among low-fat participants. Poor compliance impairs the individual health benefit, but also weakens the statistical result of entire trials. A meta-analysis on drop-out rates (see poster by Isabell Schmidt) identified several factors in the study designs, crucially influencing dietary compliance on a more global scale. It remains unclear, if these – or other factors – are predictive for individual compliance within a specific dietary trial.

Aim: Within the DiNA-P trial, we intend to elucidate predictive parameters for dietary compliance with respect to overall caloric intake and intended diet composition.

Methods: Baseline values on anthropometry, metabolism and dietary status as well as dietary information on the first treatment phase of 150 subjects within the DiNA-P trial were collected. Compliance was classified by pre-defined thresholds (caloric intake: 1200-1500 kcal; carbs in low-carb: <40 g; fat EI% in low-fat: <30 %; alcohol: full abstinence) and with a 10 % tolerance margin. Correlation analysis was used to predict binary compliance status for each dietary outcome based on screening parameters.

Results: In both diets, obesity on its own had no effect on any compliance outcome. With respect to diet-specific targets of diet composition, no baseline parameter correlated with the change of the respective dietary outcome. By cut-off analysis, we determine that low-carb compliant subjects had a significantly higher FLI and HbA1c compared to incompliant counterparts. Low-fat compliant subjects had significantly higher levels of serum lipids, inflammatory parameters (CRP, ferritin), liver fat content and lower a-priori fat than low-fat incompliant participants. Caloric restriction below 1500 kcal was managed by subjects with significantly lower baseline energy intake (both groups), significantly higher baseline TG, LDL, body fat content and energy intake (low-fat group only) as well as higher age, fibrosis score and fasting levels for insulin and c-peptide (low-carb group only).

Conclusions: In our sample, obese subjects are not specifically prone to incompliance or compliance. However, subjects with higher liver fat content showed higher compliance in both groups. Metabolic impairments, which are commonly specifically attributed to overconsumption of either fat (dyslipidemia, inflammation) or carbohydrates (hyperglycemia), were predicting successful restriction of the respective nutrient. Knowing own health risks and already aiming for a more healthy specific diet seem to be of additional motivational value for an intended lifestyle intervention.

Microcirculatory changes in the liver of patients with refractory ascites and their relationship with diabetes and alcohol

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Background & Aims: The determinants of refractory ascites have not been fully characterized. The aims of this study were to assess the liver histopathological alterations associated with refractory ascites and their relationship with comorbidities (alcohol and diabetes).

Methods: Consecutive patients with cirrhosis who underwent liver transplantation were retrospectively included. Patients' characteristics at the time of listing were analyzed. The native livers were reviewed and lesions associated with refractory ascites were examined.

Results: Out of the 89 patients included, 30 had refractory ascites, and 59 did not (including 35 without ascites and 24 with diuretic-sensitive ascites). Patients with and without refractory ascites did not differ by the amount of fibrous tissue, or the features of fatty liver disease. By contrast, microvascular changes, namely sinusoidal dilatation ($p<0.001$), diffuse perisinusoidal fibrosis ($p=0.001$), hepatic venous thromboses ($p=0.004$) and vascular proliferation ($p=0.01$) were more frequently observed in the livers of patients with refractory ascites. Diabetes (57% *vs.* 31%, $p=0.02$) and alcohol as a causal factor for cirrhosis (80% *vs.* 42%, $p=0.001$) were more frequent in patients with refractory ascites than in those without. By multivariate analysis, refractory ascites was independently associated with diabetes mellitus (odds ratio (OR) (95% confidence interval (CI)) 7.29 (1,79-29.66, $p=0.006$), alcohol as a causal factor for cirrhosis (OR (95% CI) 5.96 (1.48-24.03) $p=0.01$), higher MELD (OR (95% CI) 1.21 (1.05-1.38) $p=0.007$) and lower serum sodium (OR (95% CI) 0.87 (0.78-0.98) $p=0.02$).

Conclusions: Liver microcirculatory changes are associated with refractory ascites. Diabetes and alcohol may explain refractory ascites by causing microangiopathy.

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Alternatively activated macrophages are not implicated in control of nonshivering thermogenesis

The sympathetic nervous system (SNS) is classically recognized as the main driver of catecholaminemediated activation of nonshivering thermogenesis. Recent studies suggest that cold-induced activation of alternatively activated macrophages induces local catecholamine production to stimulate nonshivering thermogenesis. Here we report that the peripheral deletion of tyrosine hydroxylase (TH) in bone marrow of adult mice neither alters energy expenditure upon cold exposure nor stimulates markers indicative of browning in inguinal white adipose tissue (iWAT) [1]. Subsequent studies revealed that TH is absent in white and brown adipose tissue (BAT) macrophages of mice exposed to either room temperature or cold exposure (4°C). In line with these data, depletion of macrophages from iWAT and BAT primary cells had no effect on the thermogenic capacity of these cells. Treatment of iWAT and BAT primary cells with conditioned media from IL-4 stimulated bone marrowderived macrophages likewise had no meaningful effect on thermogenic pathways, including the expression of Ucp1 and Pgc-1α. In line with these data, IL-4 treatment of mice at various environmental temperatures ranging from thermoneutrality to cold exposure had no effect on energy expenditure and no difference in energy expenditure was observed between Il4ra-/- mice and their wild-type controls. In summary, our data indicate that macrophages lack TH, the key enzyme responsible to produce catecholamines, and suggest that alternatively activated macrophages do not play a major role in the regulation of nonshivering thermogenesis. [1] "Alternatively activated macrophages do not synthesize catecholamines or contribute to adipose tissue adaptive thermogenesis." Fischer et al., Nature Medicine 2017

Abstract Christina Gar
für German-French Conference 19.-20. April 2018

Patterns of Plasma Glucagon Dynamics Do Not Match Metabolic Phenotypes in Young Women

Context: The role of hyperglucagonemia in type 2 diabetes is still debated.

Objective: We analyzed glucagon dynamics during oral glucose tolerance tests (oGTTs) in young women with one out of three metabolic phenotypes: healthy control (normoglycemic after a normoglycemic pregnancy), normoglycemic high-risk (normoglycemic after a pregnancy complicated by gestational diabetes), and prediabetes/screening-diagnosed type 2 diabetes. We asked if glucagon patterns were homogeneous within the metabolic phenotypes.

Design and Setting: Five-point oGTT, sandwich enzyme-linked immunosorbent assay for glucagon, and functional data analysis with unsupervised clustering.
Participants: Cross-sectional analysis of 285 women from the monocenter observational study Prediction, Prevention, and Subclassification of gestational and type 2 Diabetes, recruited between November 2011 and May 2016.

Results: We found four patterns of glucagon dynamics that did not match the metabolic phenotypes. Elevated fasting glucagon and delayed glucagon suppression was overrepresented with prediabetes/diabetes, but this was only detected in 21% of this group. It also occurred in 8% of the control group.

Conclusions: We conclude that hyperglucagonemia may contribute to type 2 diabetes in a subgroup of affected individuals but that it is not a *sine qua non* for the disease. This should be considered in future pathophysiological studies and when testing pharmacotherapies addressing glucagon signaling.

Age-associated alterations of muscle resident progenitor cells disturb the metabolic homeostasis of skeletal muscle

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Background: Aging is associated with a progressive decline of functional and regenerative capacity of muscle tissue. Extracellular matrix (ECM) components are extrinsic factors of the stem cell niche necessary to maintain a proper regeneration and metabolic tissue function. Here, we hypothesize that age-related alterations of both fibro-adipogenic progenitor cells (FAPs) and their myogenic counterparts, the satellite cells, disturb metabolic homeostasis in muscle. Specifically, the ECM protein Periostin might play a major role in the pathological age-associated muscle disorders that contribute to impaired oxidative capacity in aging muscle.

Methods: Muscle resident progenitors cells from old, young and Periostin knockout (Postn(-/-)) mice were analyzed by Fluorescence-activated cell sorting (FACS). Specifically, FAPs from old and young mice were collected for functional and array-based gene expression analysis. Mitochondrial function was assessed from different muscles of Postn(-/-) using gene expression analysis of mRNA and protein levels and oxidative capacity.

Results: Microarray analysis of old mice FAPs expose a down-regulation of genes related with ECM. Moreover, analysis of aged and Postn(-/-) satellite cells *in vitro* showed a reduction of myogenic gene expression while adipogenic gene profile were increased in FAPs from Postn(-/-). In fact, deletion of Periostin resulted in a switch toward glycolytic fiber-types, accompanied by a reduction of oxidative capacity and mitochondrial biogenesis markers in skeletal muscle.

Conclusion: These results indicate that alterations in the local microenvironment might be associated with the fibro-adipogenic switch observed in aging muscle, and ECM -component, Periostin might play a key role in age-associated metabolic muscle dysfunction. Therefore, Periostin might serve as potential therapeutic target to preserve metabolic homeostasis during skeletal muscle aging.

Intergenerational control of metabolism by Polycomb

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Abstract

Obesity and diabetes have reached epidemic proportions worldwide, with approximately 1.9 billion overweight adults and more than 600 million clinically obese and diabetics. Obesity is known for being the main risk factor for diabetes development and, together with cardiovascular disease, being among the first causes of death in westernised countries.
The exponential rise in the incidence of diabetes and obesity worldwide, and the little contribution of GWAS studies to understanding their pathogenesis, suggest that diabetes and obesity are not classical genetic diseases. It has been indeed shown that childhood obesity and diabetes are also on the rise and parental environment and lifestyle choices influence offspring embryonic development and health trajectories. Known as Epigenetic Inheritance (EI), its discovery constitutes one of the biggest paradigm shifts in science of the recent years and holds potential in explaining the exponential rise in the incidence of diabetes and obesity. Current evidence suggests that the information stored in the germline epigenome is susceptible to the environment, resists developmental re-setting and is carried on to the following generations even in the absence of the causative event. The alteration of chromatin structure is massively involved in inter/trans-generational inheritance of obesity. Undoubtedly, sperm chromatin structure is found to be extremely dynamic and to serve as a mediator of TEI.
In mouse spermatozoa, nucleosomes are by 99% replaced by protamines during spermatogenesis and evidence exists that retained paternal histones (10% in human) provide the template for embryonic chromatin organization, embryonic development and participate to epigenetic inheritance.
Here, we show that a paternal pre-conceptional acute high-fat diet (HFD) induces glucose intolerance and insulin resistance in unexposed offspring. Mechanistically, acute HFD impinges on Polycomb Repressive Complex 2 (PRC2) activity during spermatogenesis and its genetic disruption causes germ-line transmission of Intergenerational Epigenetic Inheritance of metabolic traits with also evidence of gender specificity protection in female.
Our results provide first genetic evidence of Polycomb-dependent paternal epigenetic inheritance in mammals, in line with recently published findings on lower organisms, such as *C. Elegan*s and *D. Melanogaster*.

Lipidomic profiling reveals possible biomarkers of brown adipose tissue aging

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Adipose tissue is central to the regulation of energy balance. While white adipose tissue is responsible for triglyceride storage, brown adipose tissue specializes in energy expenditure. The age-related decline of brown adipose tissue function is thought to exacerbate the development of metabolic disease. The aim of this study was to determine changes in the lipidome composition of aging brown adipose tissue and whether these might affect the ability of brown fat-resident progenitors to give rise to thermogenic brown adipocytes. Therefore, a mass-spectrometric approach was used to identify potential lipid biomarkers of aged brown fat and cross-referenced with transcriptomic and proteomic analyses. Among the most prevalent changes were increased tissue levels of prenols. Prenol lipids are synthesized by the mevalonate pathway and can give rise to important lipid mediators, for instance quinones and dolichols. Consistent with the biomarker profiles of other tissue types, further analysis by HPLC revealed a significant age-dependent accumulation of dolichols in murine brown adipose tissue. Gene expression analysis of dolichol metabolism genes consistently revealed significant changes in aged brown adipose tissue. We further show that enzymes involved in dolichol dependent processes, such as dolichol kinase (DOLK), influence brown adipogenesis *in vitro* and *in vivo*. These findings taken together suggest that this lipid species may act as a functionally relevant biomarker of brown adipose tissue aging and represents a potential target to treat metabolic disease.

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Adropin treatment lowers hepatic glucose output *in vitro* but does not improve whole-body glucose metabolism in obese mice

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Secreted peptides have a central role in glucose homeostasis. Mice lacking the secreted peptide adropin (encoded by *Enho* gen) display increased severity of glucose homeostasis impairment and fat metabolism disorder. Furthermore, liver *Enho* mRNA expression is almost eliminated in obese animals. In humans, low circulating adropin levels are associated with obesity, markers of insulin resistance, dyslipidemia and coronary atherosclerosis. However, the impact of adropin on hepatic glucose metabolism has not yet been studied in detail.

We show that long-term adropin treatment for 24h significantly reduces forskolin- and dexamethasone-induced glucose output in human upcyte hepatocytes as well as in rat hepatoma cells (H4IIe). Furthermore, acute adropin treatment (~1h) reduces glucagon-induced glycogenolysis in primary rat hepatocytes. At the molecular level, adropin treatment did not change the expression of the key gluconeogenic enzymes *G6PC* or *PCK1*, has no effect on cAMP-activated protein kinase A (PKA) signaling, including the phosphorylation levels of cAMP-responsive element binding protein (CREB), and did not modulate the intracellular insulin signaling pathway. Taken together these data suggest a mechanism independent from the classical pathways. However, treatment of female ob/ob mice with five adropin injections (450 nmol/kg/i.p.) over 3 days did not improve oral glucose tolerance or whole-body insulin sensitivity measured by insulin tolerance test.

These data provide important insights into adropin's effects on hepatic glucose output, and provide further evidence supporting the potential of adropin in treating insulin resistance and T2D. However, unmodified adropin treatment fails to improve glucose tolerance and insulin sensitivity in obese mice. Therefore, the exact molecular mechanism including receptor identification as well as modifications of adropin to improve both, stability and potency, is required to obtain a more powerful therapeutic peptide.

Hypothalamic region-specific astrocyte regulation of glucose homeostasis and energy balance

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The hypothalamus is organized in defined neuronal nuclei that have key roles in energy balance control. Synaptic transmission and activity of these neurons are intimately regulated by astrocytes whom role in the modulation of energy metabolism have only been recently addressed. By combining pharmacogenetic approaches (DREADDs) *in vivo* and intracellular Ca^{2+} imaging (GCamP) selectively in astrocytes, we explored if specific *in vivo* Ca^{2+} manipulation in glial fibrillary acidic protein or aldehyde dehydrogenase 1 family member L1 (Aldh1L1) expressing astrocytes located in the ventromedial nucleus (VMH) or in the paraventricular nucleus (PVN) differentially participate in the control of glucose and energy homeostasis. We show that obesity leads to increased Ca^{2+} release in astrocytes in various hypothalamic nuclei and that *ex vivo* chemogenetic activation of Aldh1L1 expressing astrocytes by bath application of clozapine-n-oxide evoked a specific DREADD-dependent increase in intracellular Ca^{2+} release. Additionally, *in vivo* specific manipulation of astrocytes in the VMH induced a change in peripheral substrate utilisation and exacerbated responses to neuroglucopenia by enhancing counter-regulatory responses to 2 deoxy-glucose induced hypoglycemia. In contrast, manipulation of astrocyte in the PVN decreased glucose tolerance and energy expenditure. Finally, we provide evidence that astrocytic control of energy balance is partially mediated through adaptive change in the autonomic nervous system. In conclusion, we show that *in vivo* modulation of astrocyte populations located in discrete hypothalamic nuclei have distinct biological output onto energy homeostasis. Our data supports a concept in which obesity-associated diseases might be partially mediated through molecular and signaling changes in hypothalamic astrocytes.

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Fibre supplementation for the prevention of type 2 diabetes and improvement of glucose metabolism: the randomised controlled Optimal Fibre Trial (OptiFiT).

BACKGROUND: Insoluble cereal fibres have been shown in large prospective cohort studies to be highly effective in preventing type 2 diabetes, but there is a lack of interventional data. Our 2 year randomised double-blind prospective intervention study compared the effect of an insoluble oat fibre extract with that of placebo on glucose metabolism and incidence of diabetes.

METHODS: A total of 180 participants with impaired glucose tolerance underwent a modified version of the 1 year lifestyle training programme PREvention of DIAbetes Self-management (PREDIAS) and were randomised to receive a fibre supplement (n = 89; 7.5 g of insoluble fibre per serving) or placebo (n = 91; 0.8 g of insoluble fibre per serving) twice daily for 2 years. Eligible participants were men and women, were at least 18 years old and did not report corticosteroid or other intensive anti-inflammatory treatment, fibre intolerance or any of the following disorders: overt diabetes, chronic or malignant disease, or severe cardiopulmonary, endocrine, psychiatric, gastrointestinal, autoimmune or eating disorder. Participants were recruited at two clinical wards in Berlin and Nuthetal. The allocation was blinded to participants and study caregivers (physicians, dietitians, study nurses). Randomisation was conducted by non-clinical staff, providing neutrally numbered supplement tins. Both supplements were similar in their visual, olfactory and gustatory appearance. Intention-to-treat analysis was applied to all individuals.

RESULTS: After 1 year, 2 h OGTT levels decreased significantly in both groups but without a significant difference between the groups (fibre -0.78 ± 1.88 mmol/l [p ≤ 0.001] vs placebo -0.46 ± 1.80 mmol/l [p = 0.020]; total difference 0.32 ± 0.29 mmol/l; not significant). The 2 year incidence of diabetes was 9/89 (fibre group) compared with 16/91 (placebo group; difference not significant). As secondary outcomes, the change in HbA1c level was significantly different between the two groups (-0.2 ± 4.6 mmol/mol [-0.0 ± 0.0%; not significant] vs +1.2 ± 5.2 mmol/mol [+0.1 ± 0.0%; not significant]; total difference 1.4 ± 0.7 mmol/mol [0.1 + 0.0%]); p = 0.018); insulin sensitivity and hepatic insulin clearance increased in both groups. After 2 years, improved insulin sensitivity was still present in both groups, although the effect size had diminished. Separate analysis of the sexes revealed a significantly greater reduction in 2 h glucose levels for women in the fibre group (-0.88 ± 1.59 mmol/l [p ≤ 0.001] vs -0.22 ± 1.52 mmol/l [p = 0.311]; total difference 0.67 ± 0.31 mmol/l; p = 0.015). Levels of fasting glucose, adipokines and inflammatory markers remained unchanged in the two groups. Significantly increased fibre intake was restricted to the fibre group, despite dietary counselling for both groups. No severe side effects occurred.

CONCLUSIONS/INTERPRETATION: We cannot currently provide strong evidence for a beneficial effect of insoluble cereal fibre on glycaemic metabolism, although further studies may support minor effects of fibre supplementation in reducing glucose levels, insulin resistance and the incidence of type 2 diabetes.

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Role of the Testicular Nuclear Receptors in hepatic lipid and glucose metabolism

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Nuclear receptors are a family of ligand gated transcription factors that respond to hormones, lipid species and nutrients. They orchestrate and synchronize major biological processes such as cell division, reproduction, immune responses and metabolism.

The two related Testicular Receptors 2 and 4 are co-expressed in many tissues. The lethality of the TR2/TR4 double-knockout mice suggests that they coregulate fundamental genes. However, the exact mechanisms of action of these receptors and their target genes have not yet been identified. In order to determine the precise role of TR2 and TR4 in metabolism, we generated liver-specific knockout mice. These mice display a reduced hepatic triglyceride accumulation and steatosis on high fat diet. Furthermore, RNA-Sequencing results show deregulations of metabolic genes involved in lipid synthesis and transport. Concordantly, ChIP-Sequencing analysis finds TR4 binding sites in the promoters of these deregulated metabolic genes. Altogether, our findings point to an important role for TR2/TR4 in the regulation of key metabolic genes involved in obesity induced hepatic steatosis.

Keywords : Hepatic steatosis, Nuclear receptors, Lipid signalling.

HYPOKALORIC DIET IN TREATMENT OF METABOLIC SYNDROME IN EXOGENOUS-CONSTITUTIONAL OBESITY

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Title: Hypocaloric Diet in the Treatment of Metabolic Syndrome in Exogenously-Constitutive Obesity

Objective: To optimize diagnosis and treatment of metabolic syndrome in adolescents with exogenously-constitutional obesity

Methods: Twenty-nine adolescents with exogenously-constitutional obesity of varying severity were examined, the average age was 13.0 ± 2.6 years living in the Ferghana region of Uzbekistan. Diagnosis of the metabolic syndrome was carried out on the basis of diagnostic criteria IDF 2007. The risk of developing the metabolic syndrome was calculated using the computer program developed around the waist circumference in children and adolescents of the Uzbek population. The treatment was carried out using a hypocaloric diet developed for children and adolescents (Rakhimova GN, Azimov S.S., 2016), depending on the age, taking into account the national cuisine.

Results: In assessing the components of the metabolic syndrome of children and adolescents with exogenously constitutive obesity,WC≥90 and BMI> 97 percentiles were found in all 29 subjects. Metabolic syndrome is diagnosed in 6 (24.0%) adolescents 10-16 years with exogenously-constitutional obesity. Violation of carbohydrate metabolism (fasting glycemia> 5.0 mmol / l) was detected in 3 (10.3%) of the examined. An increase in blood pressure above the IDF criterion (SBP ≥130 mmHg DBP ≥85 mmHg) was detected in 3 (10.3%) patients. Dyslipidemia in particular: hypertriglyceridemia in 4 (13.8%), a decrease in high-density lipoproteins <1.03 mmol / l was detected in 6 (20.7%). Against the background of hypocaloric diet, after correction of diet and lifestyle, the normalization of fasting glycemia in 2 (6.9%), the level of triglycerides decreased by half, the level of high-density lipoproteins also increased 2-fold. After 3 months, all subjects had normal hemodynamic index.

Conclusions: Thus, the initial metabolic syndrome was diagnosed in 6 (24.0%), against a background of lifestyle correction and a hypocaloric diet developed in 2 of them, the metabolic syndrome was not diagnosed.

Key words: adolescents, metabolic syndrome, dyslipidemia, hypocaloric diet.

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Coordinated targeting of cold and nicotinic receptors synergistically reverses metabolic diseases

Global obesity and type 2 diabetes mellitus (T2DM) rates have reached epidemic dimensions. So far, neither conventional lifestyle therapy nor pharmacology have been sufficient to effectively and safely fight adiposity and associated comorbidities. Currently, bariatric surgery results in the most promising weight-lowering effects, but procedures, such as roux-en-y gastric bypass and vertical sleeve gastrectomy are expensive, invasive and only applicable in heavily obese patients (BMI ≥ 40 kg/m2). Therefore, there is an urgent need for safe and effective pharmacotherapy. We here report that simultaneous targeting of cold and nicotinic receptors reverses diet-induced obesity in male mice.

Historically, pharmacological activation of brown adipose tissue (BAT) thermogenesis has been progressively pursued as an anti-obesity strategy. Catecholamines, like norepinephrine and epinephrine, potently induce BAT-mediated energy expenditure; however the systemic application is associated with severe cardiovascular side effects. Cold exposure is still the most efficacious approach to recruit and activate BAT. We thus aim to pharmacologically mimic cold exposure by targeting the cold receptor transient receptor potential cation channel subfamily M member 8 (Trpm8) with the potent agonist icilin, which generates comparable metabolic benefits like cold exposure. We found that in diet-induced obese (DIO) mice, treatment with icilin enhances energy expenditure and dose-dependently lowers body weight, without affecting food intake. To further potentiate body weight loss, we aimed to add an appetite suppressant and therefore sought for the identification of an ideal pharmacological partner to icilin. To that end, we specifically targeted the nicotinic acetylcholine receptor (nAChR) subtype α3β4, which we had recognized as a potential regulator of systemic energy homeostasis. Pharmacological activation of α3β4 nAChR with a selective agonist, dimethylphenylpiperazinium (DMPP), inhibits food intake and reverses diet-induced glucose intolerance in DIO mice.

Most importantly, combinatorial targeting of Trpm8 and α3β4 nAChR orchestrates synergistic anorexic and thermogenic pathways to potently reverse diet-induced obesity, dyslipidemia and glucose intolerance in DIO mice.

Genomic glucocorticoid responses in mouse adipose tissue.

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Glucocorticoid hormones (GC) are steroid hormones involved in many physiologic and metabolic processes and their actions have been linked to metabolic syndrome. At the molecular level, GC actions are mediated through their interactions with Glucocorticoid Receptor (GR) belonging to the nuclear hormone receptor superfamily of ligand activated transcription factor.

In many tissues, GR has been shown to affect body weight, insulin sensitivity by regulating glucose and lipid metabolism. Indeed, GR is involved in adipocyte differentiation, and its deficiency affects the systemic metabolism. Because its genomic actions and its targets genes in adipose tissues remain unclear, we used NGS technics such as ChIP-Seq and RNA-Seq on mouse adipose tissues. The brown adipose tissue GR cistrome showed that GR actions are involved in fatty acid, lipid metabolic processes, response to insulin stimulus, brown fat differentiation. In addition, motif analyses of GR-bound sequences allowed the identification of potential GR co-regulators involved in the gene expression program orchestrating physiology. As metabolic syndrome constitutes a major challenge on public health, the data obtained from mice under high-fat regimen bring a better understanding of the regulation or dysregulation of signaling pathways leading to metabolic disorders.

Stefan Kabisch, Sabrina Bäther, Ulrike Dambeck, Margrit Kemper, Christiana Gerbracht, Caroline Honsek, Anna Sachno, Andreas F.H. Pfeiffer.

Liver Fat Scores Moderately Reflect Interventional Changes in Liver Fat Content by a Low-Fat Diet but Not by a Low-Carb Diet.

BACKGROUND: Non-alcoholic fatty liver disease (NAFLD) is a common metabolic disorder all over the world, mainly being associated with a sedentary lifestyle, adiposity, and nutrient imbalance. The increasing prevalence of NAFLD accommodates similar developments for type 2 diabetes and diabetes-related comorbidities and complications. Therefore, early detection of NAFLD is an utmost necessity. Potentially helpful tools for the prediction of NAFLD are liver fat indices. The fatty liver index (FLI) and the NAFLD-liver fat score (NAFLD-LFS) have been recently introduced for this aim. However, both indices have been shown to correlate with liver fat status, but there is neither sufficient data on the longitudinal representation of liver fat change, nor proof of a diet-independent correlation between actual liver fat change and change of index values. While few data sets on low-fat diets have been published recently, low-carb diets have not been yet assessed in this context.

AIM: We aim to provide such data from a highly effective short-term intervention to reduce liver fat, comparing a low-fat and a low-carb diet in subjects with prediabetes.

METHODS: Anthropometric measurements, magnetic resonance (MR)-based intrahepatic lipid (IHL) content, and several serum markers for liver damage have been collected in 140 subjects, completing the diet phase in this trial. Area-under-the-responder-operator-curves (AUROC) calculations as well as cross-sectional and longitudinal Spearman correlations were used.

RESULTS: Both FLI and NAFLD-LFS predict liver fat with moderate accuracy at baseline (AUROC 0.775-0.786). These results are supported by correlation analyses. Changes in liver fat, achieved by the dietary intervention, correlate moderately with changes in FLI and NAFLD-LFS in the low-fat diet, but not in the low-carb diet. A correlation analysis between change of actual IHL content and change of single elements of the liver fat indices revealed diet-specific moderate to strong correlations between ΔIHL and changes of measures of obesity, ΔTG, and ΔALT (all low-fat, only) and between ΔIHL and ΔGGT (low-carb, only). With exception for a stronger decrease of triglycerides (TG) levels in the low-carb diet, there is no statistically significant difference in the effect of the diets on anthropometric or serum-based score parameters.

CONCLUSION: While liver fat indices have proved useful in the early detection of NAFLD and may serve as a cost-saving substitute for expensive MR measurements in the cross-sectional evaluation of liver status, their capability to represent interventional changes of liver fat content appears to be diet-specific and lacks accuracy. Liver fat reduction by low-fat diets can be monitored with moderate precision, while low-carb diets require different measuring techniques to demonstrate the same dietary effect.

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REGISTRATION: *NCT02609243*

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Reduced intake of branched-chain amino acids reduced intake does not improve insulin sensitivity in overweight patients with type 2 diabetes

Serum levels of branched-chain amino acids (BCAA: valine, leucine, isoleucine) are elevated in patients with insulin resistance and/or type 2 diabetes (T2D). BCAA intake improves mitochondrial function in young healthy adults, however, in middle aged patients with T2D this association is unclear. On the other hand, BCAA lower the expression of the hepatokine fibroblast growth factor 21 (FGF21), which enhances the glucose uptake in adipose tissue. We tested the hypothesis that dietary reduction of BCAA improves insulin sensitivity (IS) and mitochondrial efficiency and increases FGF21 levels in patients with type 2 diabetes.

In a randomized, placebo-controlled, double-blinded study 12 patients (8 male, 4 female; age 54.0±3.9 years, BMI 30.8±2.8 kg/m^2, HbA1c 6.6±0.9%, 49±10 mmol/mol) with T2D duration <5 years, received a 4-week isocaloric diet with a constant protein uptake of 1 g/kg body weight.

In a cross-over design, the diet contained either the complete set of amino acids (BCAA$^+$) or 60% less BCAA (BCAA$^-$) for one week, separated by a one-week wash-out period. Whole-body IS was assessed by hyperinsulinemic-euglycemic clamps (HEC) and beta-cell function using mixed-meal tolerance tests (MMT). Mitochondrial efficiency was assessed by respiratory control ratio (RCR) using high-resolution respirometry in muscle and adipose tissue biopsy samples. FGF-21 serum levels were quantified by ELISA.

The IS was lower after $BCAA^-$ than after $BCAA^+$ diet (3.1 ± 1.7 vs. 3.5 ± 1.8 $mg*kg^{-1}*min^{-1}$, $p < 0.01$). However, this difference disappeared after correction for the prevalent insulin levels during HEC. The secretion of insulin and C-peptide was 20% lower after $BCAA^-$ diet (iAUC insulin: 21 ± 11 vs 29 ± 19 $mU*ml^{-1}*4h^{-1}$, $p < 0.01$). After $BCAA^-$ diet, RCR was 1.7-fold higher in adipose tissue ($p<0.05$) but unchanged in skeletal muscle. The $BCAA^-$ diet increased FGF-21 levels from 323 ± 189 to 405 ± 233 pg / ml, $p<0.05$).

In conclusion, a dietary reduction of BCAA by 60 % for 1 week results in lower stimulated insulin and C-peptide levels, higher mitochondrial efficiency in adipose tissue, but fails to improve whole body IS in T2D.

Margrit Kemper / Stefan Kabisch / Andreas F.H. Pfeiffer

Immediate and Long-term effects on Incretin release by Artificial Sweeteners – the ILIAS trial

Background: Artificial sweeteners (AS) are widely used non-caloric substitutes for sugar. They are abundant in so-called “light” soft drinks, but also in food and beverages containing sugar or other carbohydrates. AS represent a variety of chemical compounds, rendering different properties regarding sweet taste intensity, presence, absence or quality of bitter by-taste as well as intestinal digestion and/or absorption. In recent years, several epidemiological studies have highlighted, that AS may not serve helpful in the prevention or treatment of obesity but may even increase the risk for overweight, NAFLD and type 2 diabetes. However, data is highly inconclusive and more research on population scale, but also on the relevant mechanisms is needed. As one potential way of action, effects on incretin release have been discussed, but interventional data is also highly inconclusive. Cell experiments and rodent studies imply, that AS may elicit incretin release on their own; human studies mostly could not replicate those findings. Reasons for inconsistencies may be found in cohort structure, way of application or other experimental co-factors. As one of those studies, our own SeGaTRoM trial reported a significant increase of GIP secretion after combined saccharin-glucose ingestion compared to glucose alone. Effects on insulin, c-peptide, GLP-1 or PYY were not found.
Objective: The ILIAS trial therefore intended to replicate the findings from SeGaTRoM, to gather more data on different types of AS and to investigate both short- and long-term effects. Methods: We conducted separate oral stimulation tests with saccharin, aspartame and sucralose, both alone and in combination with glucose in 14 healthy men and analysed serum levels for glucose, insulin, c-peptide, GIP, GLP-1 and
GLP-2 (ILIAS-1). Also, 10 healthy men underwent a 4-week exposure with unsweetened and saccharine-sweetened non-caloric soft drinks in a cross-over design and were tested with conventional oGTTs before and after the intervention phases (ILIAS-2).Results: There were no statistically significant differences in insulin secretion or incretin release comparing glucose with glucose-combined AS ingestion. AS alone did not elicit incretin release. 2-hours glucose levels were significantly lower under all kinds of AS compared to glucose ingestion.
Long-term exposure with saccharin did not affect glucose or hormone levels.Conclusion: We were unable to replicate findings on hormone secretion by AS intake from the SeGaTroM trial, but found similar glucose curves. Also, saccharine, aspartame and sucralose did not differ in their biological effects in short-term. We cannot support hypotheses on detrimental long-term effects of saccharin.

Deletion of the mammalian Indy homolog (Slc13a5) in neurons increases energy expenditure in mice

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INDY (I'm Not Dead Yet) is a transporter of TCA cycle intermediates, mediating cellular citrate uptake and is highly expressed in liver and brain. Reduced expression of *Indy* in lower organisms extended lifespan, reduced whole body fat content and increased mitochondrial biogenesis. In mammals, whole body deletion of the mammalian *Indy* homolog (*mIndy*, *Slc13a5*) increased energy expenditure and protected mice from diet and aging induced obesity and insulin resistance. Here, we addressed the role of the neuronal *mIndy* in energy homeostasis. Therefore, we generated a neuron specific *mIndy* knockout (NINKO) mouse model crossing *mIndy*-floxed to NestinCre mice. Interestingly, deletion of *mIndy* in neurons led to an increase in energy expenditure compared to NestinCre mice (average 24h EE: NestinCre: 11.49±0.51 kcal/h/kg lean mass; NINKO: 13.59±0.55 kcal/h/kg lean mass; $p \leq 0.001$) after 8 weeks of high fat diet (HFD) feeding. The respiratory ratio changed from 0.78±0.007 in NestinCre mice to 0.77±0.006 in NINKO mice during light phase ($p \leq 0.001$) indicating augmented lipid oxidation in NINKO mice. Body temperature increased significantly in NINKO compared to NestinCre mice (NestinCre: 37.03 ± 0.09°C NINKO: 37.29 ± 0.05°F at night time; $p \leq 0.05$). While body weight was only reduced numerically, fat mass was reduced and lean mass increased significantly in NINKO mice (NestinCre: 59.95 ± 0.72% lean mass, 30.58 ± 0.85 fat mass; NINKO: 62.71 ± 0.70% lean mass, 27.39 ± 0.87% fat mass after 8 weeks of HFD feeding; $p \leq 0.05$). Hyperinsulinemic-euglycemic clamp studies showed improved insulin sensitivity in NINKO mice (GINF NestinCre: 26.2 ± 1.9 mg/kg/min; NINKO: 33.6 ± 2.4 mg/kg/min; $p \leq 0.05$). Together, these data suggest that neuronal *mIndy* is a critical regulator of energy and glucose homeostasis in mammals. Further studies will address the mechanisms involved in the effect.

Elena Lalama, Stefan Kabisch, Ulrike Dambeck, Caroline Honsek, Christiana Gerbracht, Anna Sachno, Andreas F.H. Pfeiffer

Comparison of dietary intervention strategies - OptiFiT placebo group vs. DiNA-P low-fat group

Background: Low-fat diets are effective treatments in the prevention (and therapy) of T2DM. However, the individual approach of consultation may differ and might influence the long-term effectiveness of the intervention. In Germany, the German Society for Nutrition (DGE) specifically recommended low-fat diets until early 2018. DGE dietitians provide the opportunity for consultation in various intervention styles, such as PREDIAS, a systematic group-based approach or single consultation. It is unknown, if PREDIAS is superior to individual treatment.

Aim: We intend to compare two one-year low-fat dietary treatments from different studies, but with comparable cohorts.

Methods: Subjects were selected from the low-fat treatment regime of DiNA-P (currently n=117) and the placebo low-fat arm of OptiFiT (n=91). Consultation in DiNA-P was conducted individually for each participant, while the OptiFiT intervention was based on PREDIAS (group consultation). For detailed study designs, please see the according posters. For further alignment of the cohorts, only DiNA-P subjects from the conventional intervention group with baseline IGT were selected. 50 subjects per study (each 23 male) were compared with respect to improvements in body weight, body composition, lipid profile, blood pressure, liver enzymes and fatty liver indices. Per-protocol analysis was conducted.

Results: After 6 months, the DiNA-P approach is significantly more successful with regard to body weight, waist-to-hip-ratio, body-fat content, uric acid, triglycerides, LDL, HbA1c, NEFA and FLI. After 12 months, DiNA-P low-fat subjects still improved significantly stronger with respect to leukocyte count, IHL and NEFA.

Conclusions: An individual approach in dietary consultation may clearly serve beneficial in the first months of treatment, but after one year only few differences between the two strategies can be found. Individual responsiveness to consultation stimuli alone or currently unknown compliance factors may mainly contribute to success or failure within a dietary treatment.

Current Research Abstract – Lassi Maximilian 15.3.2018

Intergenerational inheritance of circadian arrhythmia in mice

Humans – as well as most other life on Earth – possess an endogenous circadian system that optimally synchronizes physiology and behavior to the solar day. This alignment is crucial for proper metabolic control and can be seen from the fact that in the last decades through industrialization the daily routine of many humans has slowly shifted more and more towards working and eating at night. This has lead to disruptions in the synchronization of the circadian clock. Different cohort studies showed that humans working in shift work showed a higher risk of developing glucose intolerance, obesity and eventually type-2 diabetes.
The link between a disrupted circadian rhythm and metabolic phenotypes raises the question if these environmental exposures that affect our current generation can also be epigenetically inherited and may also lead to metabolic phenotypes in the offspring.

The aim of my current PhD project is to investigate the extent to which circadian arrhythmia has an effect on the metabolism, to which extent this effect is passed on to unexposed offspring and the molecular mechanisms behind it.
To achieve this goal I restricted the food intake of C57BL/6J male mice for 30 days to the resting light phase between 6am and 6pm. This setup mimics the resting time for humans, in which they would normally sleep most of the time. After the 30 days of restricted feeding I mated the restricted C57BL/6J mice with native wildtype females. The phenotype of the F1 generation was examined. Compared to control matings, these offspring showed higher food intake in the resting phase, higher glucocorticoid stress levels and higher blood glucose values in the male offspring compared to control mice. Interestingly the female offspring do not show these phenotypes. The influence of circadian arrhythmia on future generations therefore is most likely a sex-dependent inherited phenotype. It also shows that even a short time period of environmental exposure has a significant effect on the metabolism of following generations.
Our overall goal is to determine the underline mechanisms for these findings. Our findings support the theory that changes in glucocorticoid levels in seminal fluid of the fathers can impact the early stages of embryogenesis and with that lead to phenotypes in the offspring mature mice.

Effects of a low carbohydrate versus a low-fat formula diet on glycaemic metabolism and liver fat content in individuals with overt Type 2 Diabetes (DiNA-D study)

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Background – Overweight and obesity, hypertension, dyslipidaemia as well as hepatic steatosis are metabolic dysfunctions that correlate closely with the nowadays substantial chronic disease of Type 2 Diabetes (T2D). People suffering from the aforementioned conditions are significantly more affected by cardio- and microvascular impairments, e.g. retinopathy and neuropathy. An impaired glucose metabolism is connotatively modifiable by lifestyle factors, such as diet and physical activity. According to recent data, a diet low in carbohydrates shows considerable metabolic improvements in patients with T2D, compared with the traditional recommendation for a low-fat diet. A specific focus on PUFAs is required, as the Mediterrean diet - being the most recommendable diet - is moderately low in carbohydrates, but high in PUFAs.

Aims – This study aims to investigate the effects of a two-phase (3 weeks and 11 months) dietary intervention either of a low carbohydrate or a low-fat diet, with or without long-term supplementation with PUFAs.

Methods – 90 subjects (44 women), aged 34-78, mean BMI 32,6 kg/m², and overt with T2D, participated this study. Individuals were randomised according to the study design and underwent phenotyping by anthropometry, metabolic tests and body imaging. Alterations of the pre- and post-diet states were analysed.

Results – Dietary intervention improved anthropometric parameters and HbA1c levels in all groups. In the first phase, subjects on a low-fat formula diet showed significantly greater benefits regarding glycaemic metabolism and body weight. In the second phase, statistically significant differences between the four groups with respect to body weight, body composition, blood pressure, lipid levels, IHL, inflammation or neuropathy could not be detected.

Conclusion – In the long-term, different strategies in patients with overt T2DM seem to result in metabolic equipoise. Further analyses will focus on dietary compliance, safety parameters and organ-specific effects on a molecular basis.

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Improved beta-cell function of human pancreatic microislets

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Introduction: Individual islets vary in size and endocrine cell composition and consequently in hormone secretion. A standardization of cell number and composition would therefore facilitate high throughput testing of islet cell function. Dissociation of islets into single cells is accompanied by a loss of regulated insulin secretion. Dissociated islet cells have the aptitude of reaggregation. This behavior makes it possible to form microislets of an identical α:β:δ-cell ratio and a defined cell number. The present study aims to compare insulin secretion of isolated human islets and microislets from the same donors.

Material and methods: Human pancreatic islets received from ECIT Centers were either cultured overnight under standard conditions or reaggregated into microislets after digestion with trypsin. Reaggregation of islet cells was performed using the hanging-drop method. Isolated islets and microislets were tested in parallel for insulin secretion.

Results: Insulin content of isolated human islets from four donors was 23.0±1.2 ng/islet. Insulin secretion was 3.2±0.6% of content at 2.8 mM glucose and 4.6±0.8% at 12 mM glucose. Forskolin, 5 µM, increased secretion to 6.6±1.2%, palmitate, 0.6 mM, to 6.1±0.8%. Microislet aggregates of 1000, 2000 and 4000 cells contained 1.6±0.2, 2.7±0.3, 10.1±1.4 ng insulin/microislet, respectively. Basal secretion was 1-2% of content. Glucose stimulated insulin secretion 13-, 10- and 9-fold in microislets formed out of 1000, 2000 and 4000 cells, respectively.

Conclusion: Microislets display improved glucose-induced insulin secretion suggesting that beta-cells maintain glucose-responsiveness even after isolation and reaggregation. Thus, standardized microislets of human donors are suitable for functional tests.

A HUMANIZED MOUSE MODEL TO STUDY TYPE 1 DIABETES

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Type 1 Diabetes is an autoimmune disease resulting to a massive destruction of pancreatic islets β-cells by auto-reactive $CD8^+$ T-cells leading to hyperglycaemia. This multifactorial pathology implicates many genetics factors, in particular HLA molecules acting as susceptibility or protecting factors and determining islet cell auto-immunity. Despite all the evidence pointing to insulin as a key autoantigen, attempts to modulate the autoimmune response to beta-cells using insulin via different routes, although efficient in the NOD mouse, have failed in the human. New preclinical models of T1D will be required to help advancing both aims. By combining expression of HLA-A*0201 and HLA-DQ8, two molecules of predisposition of T1D, and human preproinsulin as an autoantigen, we create a new humanized mouse model to study T1D. YES mice remain insulitis- and diabetes-free up to one year of follow up, maintain normoglycemia to an intraperitoneal glucose challenge in the long-term range, have a normal β-cell mass and show normal immune responses to conventional antigens. When challenge with polyInosinic-polyCytidylic acid, YES mice develop diabetes with the presence of an insulitis. Characterization of PPI epitopes recognized by $CD8^+$ and $CD4^+$ T-lymphocytes upon immunization against human preproinsulin or along diabetes development demonstrates that the YES mice are a relevant model to study autoantigen presentation in the T1D model.

Insulin and muscle contraction regulate TBC1D1 through phosphorylation and interaction with the cytosolic tail of insulin-regulated aminopeptidase

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Abstract

In skeletal muscle, the ~1200 aa Rab GTPase-activating (GAP) protein TBC1D1 is phosphorylated by AKT and AMPK in response to insulin and contraction. Genetic ablation of Tbc1d1 or mutation of phosphorylation sites for AKT and AMPK impairs GLUT4 translocation from storage vesicles (GSVs) to the plasma membrane. However, the exact phosphorylation pattern and the mechanism how the signal is transmitted to GSVs is unclear. In this study, we studied the phosphorylation of TBC1D1 and its impact on TBC1D1's catalytic activity. Therefore, we cloned, expressed and purified recombinant full-length TBC1D1 in Sf9 insect cells via the Baculovirus system. Size-exclusion chromatography of the purified protein reveals a molecular mass of approx. 600 kDa, consistent with formation of TBC1D1 trimers. Full-length TBC1D1 shows RabGAP activity towards GLUT4-associated Rab8a, Rab10 and Rab14 but with a 200-fold increase in velocity compared to the 50 kDa GAP domain expressed in E. coli. We mapped the phosphorylation sites of TBC1D1 via mass spectrometry and phospho-specific antibodies. Our results show that full-length TBC1D1 is phosphorylated at Ser^{231} in response to AMPK and at Thr^{590} in response to both AMPK and AKT. While in vitro phosphorylation of TBC1D1 by AKT or AMPK increased 14-3-3 binding, it did not alter the RabGAP activity. However, we found that full-length TBC1D1 interacts with the 110 aa cytoplasmic domain of insulin-regulated aminopeptidase (IRAP), a resident protein in GLUT4 storage vesicles, and this binding is disrupted by phosphorylation of TBC1D1 by AKT or AMPK. Our data indicate that insulin and contraction-mediated activation of AKT/AMPK regulates recruitment of TBC1D1 to GSVs via phosphorylation and interaction with IRAP.

GNIP1 E3 ubiquitin ligase is a novel player in regulating glycogen metabolism in skeletal muscle

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ABSTRACT

Glycogenin-interacting protein 1 (GNIP1) is a TRIM protein with E3 ubiquitin ligase activity that interacts with glycogenin. These data suggest that GNIP1 could play a major role in the control of glycogen metabolism. However, direct evidence based on functional analysis remains to be obtained. The aim of this study was 1) to define the expression pattern of glycogenin-interacting protein/Tripartite motif containing protein 7 (GNIP/TRIM7) isoforms in humans, 2) to test their ubiquitin E3 ligase activity, and 3) to analyze the functional effects of GNIP1 on muscle glucose/glycogen metabolism both in human cultured cells and *in vivo* in mice. We show that GNIP1 was the most abundant GNIP/TRIM7 isoform in human skeletal muscle, whereas in cardiac muscle only TRIM7 was expressed. GNIP1 and TRIM7 had autoubiquitination activity *in vitro* and were localized in the Golgi apparatus and cytosol respectively in LHCN-M2 myoblasts. GNIP1 overexpression increased glucose uptake in LHCN-M2 myotubes. Overexpression of GNIP1 in mouse muscle *in vivo* increased glycogen content, glycogen synthase (GS) activity and phospho-GSK-3α/β (Ser21/9) and phospho-Akt (Ser473) protein content, whereas decreased GS phosphorylation in Ser640. These modifications led to decreased blood glucose levels, lactate levels and body weight, without changing whole-body insulin or glucose tolerance in mouse. In conclusion, GNIP1 is an ubiquitin ligase with a markedly glycogenic effect in skeletal muscle.

4 D imaging of insulin secretory granule dynamics and secretion in primary beta cells with lattice light sheet microscopy

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Total internal reflection microscopy (TIRFM) has been the method of choice for many years to image insulin secretory granule (SG) dynamics and secretion in primary beta cells and insulinoma cell lines. However, it only allows for imaging of SGs located <200 nm from the surface of the cell attached to the glass, thereby restricting the view only to events happening on one side of the cell. Since beta cells have a polyhedral shape with a diameter of several µm, by TIRFM imaging events happening in the major part of the cell remain invisible. Furthermore, prior to TIRFM imaging pancreatic islets are usually dissociated into single cells – a procedure that affects cell-to-cell interaction and signaling.

These limitations can be overcome with novel microscopy techniques that allow for imaging insulin SGs within primary beta cells of isolated islets at sub-cellular resolution and high speed. Specifically, we have used lattice light sheet microscopy (LLSM) to resolve insulin SGs, which have a mean diameter of 250 nm, and for fast TIRFM-like sectioning of cells in 3 dimensions with low photo-toxicity. In this way we could image SNAP-labelled insulin SGs in isolated SOFIA (Study of Insulin Ageing) mouse islets within the whole cell volume. Use of a novel pH-sensitive SNAP-substrate further enabled us to image insulin SGs undergoing exocytosis. Hence, this is the first report ever for the use of LLSM in a primary mouse tissue at sub-cellular resolution in order to address insulin SG turnover within whole beta cells. Ultimately, this approach might be exploited to study peptide hormone turnover in other model systems, thus providing novel insights into the physiology of regulated secretion in health and disease.

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Pregnancy promotes compensatory adaptations in pancreatic alpha-cells in mice

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During pregnancy, the mother undergoes major hormonal and metabolic changes to meet the energy requirements of the growing fetus. Therefore, important functional and structural adaptations occur in the pancreatic beta cells to maintain an optimal glucose homeostasis. These adaptations arise to compensate for the increased insulin resistance. However, little is known about the adaptations of alpha cell during pregnancy. Based on the recent findings of our group, which show alpha-cell adaptations in another model of insulin resistance, i.e. obesity, we decided to investigate the morphofunctional features of pancreatic alpha-cells during pregnancy in mice.

Our groups of study are non-pregnant female mice, as control group, and pregnant mice at gestational days G12.5, G15.5 and G18.5. Non-fasting and fasted plasma glucagon and insulin concentrations were determined by ELISA. Glucagon secretion and content measurements were performed with freshly isolated islets using three different conditions: 0.5mM glucose, 11mM glucose and 0.5mM glucose + 10nM insulin. To study alpha-cell size, area and mass we used paraffin embedded pancreas sections stained for glucagon and analyzed using Metamorph Analysis Software. Proliferation assay was performed by immunohistochemistry using an antibody against glucagon and Ki67, a protein expressed among proliferating cells and absent in quiescent cells. To analyze the percentage of apoptosis we used the TUNEL Assay.

We have observed a significant reduction in non-fasting plasma glucagon levels at gestational days G12.5 and G18.5. After 8 hours of fasting, no differences in glucagon plasma levels were observed between control and G18.5 groups, although G18.5 mice were hyperinsulinemic compared to control mice. *In vitro* glucagon secretion was significantly diminished at low glucose levels in the G18.5 group comparing to the control. Regarding morphological adaptations, we have observed an increase in the alpha-cell size, area and mass in G18.5 mice. Additionally, the percentage of alpha cell proliferation was higher in G18.5 compared to control mice, and no differences in the percentage of apoptosis were detected. These findings indicate that pancreatic compensatory adaptations during pregnancy may also involve pancreatic alpha-cells.

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Intermittent fasting reduces lipid accumulation in the pancreas and prevents diabetes in NZO mice.

In obese patients, visceral fat is accompanied by ectopic fat deposition in non-adipose tissue organs such as the liver, the muscle but also the pancreas. In mice and human, fat in the pancreas is mainly stored in adipocytes and is associated with insulin resistance and β-cell dysfunction [1]. New Zealand Obese (NZO) mice develop type 2 diabetes in response to obesity. They display hyperglycemia and β-cell loss following a caloric-enriched diet with a high accumulation of adipocytes in the pancreas over the time. Intermittent fasting (IF) has been shown to have positive effects on diabetes susceptibility in animals and humans. IF also positively impacts the liver of NZO mice, but the effects on the pancreas of these animals remain unknown. NZO mice under high-fat diet were fasted every other day (IF) and compared to ad libitum (AL) fed control mice. Five weeks of IF reduced pancreas triglycerides concentration, while insulin content was similar between AL and IF. After 10 weeks of intervention, 43% of AL mice were diabetic whereas none IF developed diabetes. IF mice tended to have less adipocytes in the pancreas compared to AL non-diabetic ones, whereas AL diabetic animals displayed the lower rate of fat in the pancreas. The amount of lipid droplets in the islets was similar between IF and AL non diabetic animals while in AL diabetic mice almost no lipid droplets were visible in the islets. Our data show that IF reduces fat deposition in the pancreas and prevent diabetes development in NZO mice fed a high-fat diet, at least after 10 weeks of intervention.

1. Yu, T.Y. and C.Y. Wang, Impact of non-alcoholic fatty pancreas disease on glucose metabolism. J Diabetes Investig, 2017

Reconstructing human pancreatic differentiation by mapping specific cell populations during development.

Abstract :

Information remains scarce on human development compared to animal models. Here, we reconstructed human fetal pancreatic differentiation using cell surface markers. We demonstrate that at 7weeks of development, the glycoprotein 2 (GP2) marks a multipotent cell population that will differentiate into the acinar, ductal or endocrine lineages. Development towards the acinar lineage is paralleled by an increase in GP2 expression. Conversely, a subset of the GP2+ population undergoes endocrine differentiation by down-regulating GP2 and CD142 and turning on NEUROG3, a marker of endocrine differentiation. Endocrine maturation progresses by up-regulating SUSD2 and lowering ECAD levels. Finally, in vitro differentiation of pancreatic endocrine cells derived from human pluripotent stem cells mimics key in vivo events. Our work paves the way to extend our understanding of the origin of mature human pancreatic cell types and how such lineage decisions are regulated.

Authors:

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Primary cilia regulate transferrin endocytosis and iron homeostasis

M. Julia Scerbo, Francesco Volta, Anett Seeling, Nils O'Brien, Jantje M. Gerdes

Primary cilia are sensory organelles present in approximately 80% of cells in the adult body plan. Ciliary dysfunction causes a subset of human diseases called ciliopathies. Obesity is common clinical feature in ciliopathies like Bardet–Biedl Syndrome (BBS). BBS displays high co-morbidity with T2DM and metabolic defects. Pancreatic β-cells are ciliated and we found that IR-A is localized to the primary cilium in response to insulin. Ciliary dysfunction in BBS4 mice impairs first phase insulin release resulting in mild glucose intolerance, while ablation of primary cilia specifically in β-cells in adult mice leads to blunted insulin secretion and severe glucose intolerance due to misregulation of the Eph endocytosis and endosomal processing.

We investigated the role of primary cilia in transferrin uptake, a gold standard for the study of receptor-mediated endocytoses and an essential component for iron transport and absorption in all cell types. We test the endocytic capacity of transferrin in three different ciliary knockdown stable cell lines (β-cells, endothelial and kidney cells) and found that it was significantly decreased compared to control group, indicative of a role for primary cilium in iron homeostasis. Next we tested the iron binding capacity in BBS4 global knockout mice and observed that it was significantly reduced compared to their wild type litter mattes.

These data suggest that primary cilia are required for iron homeostasis by modulating transferrin endocytosis.

Factors of Dietary Compliance in Intervention Studies Comparing Low-carb and Low-fat Diets - a Meta-Analysis Focussing on the Drop-out Rate

Isabell Schmidt, Stefan Kabisch, Andreas F.H. Pfeiffer

Background: Low-carb and low-fat diets are main strategies for prevention and therapy of T2DM. Despite similar long-term effects on weight reduction, low-fat diets have been proven inferior with regard to certain aspects of metabolic response, but also with respect to dietary compliance, which might explain some of the missing benefit. Poor compliance affects individual health, but also impairs validity and reliability of clinical dietary trials.

Aim: In order to improve the quality of future studies by achieving lower drop-out rates, we aim to identify factors of dietary compliance from already published intervention trials.

Methods: Based on the available literature on low-carb-vs.-low-fat RCTs we conduct a meta-analysis on the drop-out rate. This parameter represents the compliance of study participants most objectively, as other data on dietary intake and even dietary aims are often somehow missing or imprecise. We evaluate correlations between the drop-out rate and factors of the study design (cohort size, study duration), cohort structure (age, sex ratio, BMI, concomitant diseases, smoking, diabetes prevalence) and study intervention (dietary aims, consultation intensity etc.) as well as other markers of compliance (data from dietary protocols). Our analyses is conducted on all selected studies together (n=42) and additionally split for intervention duration (up to 16 weeks; n=16; 17-26 weeks; n=17; 52-64 weeks (n=15)). Also, a cut-off analysis was performed.

Results: The drop-out rate is correlated with higher BMI. Age, sex, smoking and other biological factors do not seem to affect compliance. Furthermore, low target values for caloric intake, especially regarding protein and carbohydrate sources, a higher actual caloric intake and a high frequency of dietary protocols are linked to a higher drop-out rate. Financial supports for subjects and additional consultation offers were without statistically significant effect on dietary compliance. Stratification by intervention duration and the cut-off analysis did not reveal additional significant results.

Conclusions: The drop-out rate does reliably represent dietary compliance. Study cohorts with higher average BMI appear to be less compliant, also, very strict dietary targets and the individual effort for dietary protocols are main factors for compliance rather than the variety of consultation offers.

Preclinical trials to treat type 2 diabetes using NMDA receptor antagonists

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We recently demonstrated that pharmacological inhibition of pancreatic *N*-methyl-*D*-aspartate receptors (NMDARs) with the over-the-counter NMDAR antagonistic drug dextromethorphan (DXM) and its active metabolite dextrorphan (DXO) leads to antidiabetic effects in mice and men, but without the risk of hypoglycemia. Furthermore, it promotes islet cell survival under diabetogenic conditions, both *in vitro* and *in vivo* and thus delays diabetes progression *in vivo*. The most commonly prescribed antidiabetic drugs are not able to halt progressive β-cell dysfunction and death. Therefore, drugs are needed that promote islet cell survival to delay, or even stop disease progression. DXM and DXO are able to pass the blood-brain barrier (BBB) and thus antagonize central NMDARs. Therefore, they can induce undesirable central nervous side effects (e.g. fatigue and dizziness). For this reason, we designed and synthesized modifications of DXO that prevent excessive compound distribution to the central nervous system (CNS) and thus central nervous side effects on the one hand, but maintain (or increase) their antidiabetic effects on the other hand. Newly synthesized modifications of DXO were tested for their insulinotropic and blood glucose lowering effect (both *in vitro* and *in vivo*), as well as for their distribution to the CNS (*in vivo*) and their behavioral effects in mice by using different neurological tests. Compared to DXO, we were able to reduce BBB passage to a minimum of 12% and thus reduce neurological impairment of mice, but without losing the antidiabetic effects. Our data indicate that novel NMDAR antagonists can be generated by chemical modification of DXO and that these peripherally restricted antagonists of the receptor could serve as a new class of antidiabetic drugs.

MEA-based parallelized screening system for intact islets of Langerhans

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Abstract

Electrical activity of beta-cells in intact islets of Langerhans consists of membrane potential (Vm) oscillations that couple blood glucose concentration to insulin release. Oscillations are characterized by depolarized phases with action potentials and silent interburst phases. For analysis the percentage of burst activity is calculated and referred to as fraction of plateau phase (FOPP). We recently demonstrated that these oscillations can be measured non-invasively from mouse and human islets as extracellular field action potentials with microelectrode arrays (MEAs) providing an innovative and powerful tool to investigate beta-cell physiology [1, 2, 3].

Thus, the MEA technology opens a new route to 1) support the development of new drugs for the treatment of type-2 Diabetes mellitus (T2DM), and to 2) elucidate beta-cell pathophysiology e.g. during the progression of T2DM.

Until now techniques were restricted to a single electrophysiological recording per experiment. In order to meet the requirements necessary for drug screening and profiling, we developed a MEA-based high-throughput system for the recording of acute intact islets of Langerhans, the ***BetaScreen***.

In addition, to addressing the long-term effects of a diabetes-promoting environment on islets of Langerhans' functions (e.g. to mimic the development of T2DM) monitoring of electrical activity of the islets over several days or weeks is required. However, so far, all current electrophysiological techniques have been limited to short-term recordings. Here we show that we are able to ***cultivate intact islets of Langerhans on MEAs*** for more than 34 days.

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LC-Q-TOF-MS-analysis for quantification of thyroxine and metabolites in placenta

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Thyroid hormones (TH) of maternal origin are critical for the proper fetal development, especially during early pregnancy. Even minor changes in maternal TH circulation can lead to various adverse outcomes [1, 2]. Recent studies found that the metabolites of thyroxine (T_4) also play an important physiological role. For example, 3,5-diiodo-L-thyronine (T_2) and 3,3´-diiodo-L-thyronine (rT_2) can suppress the thyroid stimulating hormone (TSH) level and increase the resting metabolic rate [3]. 3-iodothyronamine (T_1AM) administration in mice leads to a hypometabolic state [4]. These metabolites may have potential influences on the fetal development. Having the capacity to make a comprehensive analysis of T_4 as well as the metabolites in placenta provides a diagnostic tool for the placental TH homeostasis. Routine TH assessment has long been achieved by measuring T_4, triiodo-L-thyronine (T_3), and TSH in blood using immunoassay (IA) method, which is of high sensitivity, but is prone to nonspecific interferences [5]. Methods based on liquid chromatography-mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS) showed better accuracy and reliability [6,7]. In this study, we report a method for the determination of T_4, T_3, 3,3´,5´-triiodo-L-thyronine (rT_3), T_2, rT_2, 3-iodo-L-thyronine (T_1), and T_1AM in placenta. The method was optimized using isotope (^{13}C-T_4, ^{13}C-T_3, ^{13}C-rT_3, ^{13}C-T_2) dilution methodology and determined by liquid chromatography quadrupole time-of-flight-mass spectrometry (LC-Q-TOF-MS). The calibration ranges from 0.5 to 150 ng mL^{-1} with R^2 values > 0.99. The method detection limits (MDLs) and the method quantification limits (MQLs) based on fresh weight were 0.01 – 0.2 ng g^{-1} and 0.04 – 0.7 ng g^{-1}, respectively. The spike-recoveries for THs (except for T_1 and T_1AM) were between 81.0% and 112%,

with a coefficient of variation (CV) of 0.5 – 6.2%. The intra-day CVs and inter-day CVs were 0.5% – 10.3% and 1.19% – 8.88%, respectively. The method was adopted for TH measurement in human and mouse placenta. The concentrations of T_4, T_3, rT_3, and T_2 were 22.9 – 35.0 ng g^{-1}, 0.32 – 0.46 ng g^{-1}, 2.86 – 3.69 ng g^{-1}, and 0.16 – 0.26 ng g^{-1} in human placenta, and 2.05 – 3.51 ng g^{-1}, 0.37 – 0.62 ng g^{-1}, 0.96 – 1.3 ng g^{-1}, and 0.07 – 0.13 ng g^{-1} in mouse placenta, respectively. The presence of T_2 was tracked in placenta tissue for the first time, indicating improved selectivity and sensitivity of our method. The validated method allows comprehensive evaluation of total T_4 and metabolites in placenta.

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Inhibition of Ceramidesynthase-6 as a Novel Therapeutic Approach to treat Obesity and Type 2 Diabetes

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High fat diet delivers surplus saturated fatty acids to non-adipose tissues, which couples with increased production of ceramides that contribute to development of insulin resistance and type 2 diabetes as amelioration of ceramide biosynthesis improves the metabolic phenotype. However, as sphingolipids are involved in multiple fundamental cellular processes, concerns exist about possible risks or adverse effects for treatment of chronic diseases. Therefore inhibiting the specific ceramide species, which are pathogenic offers new strategies for pharmaceutical intervention. In this perspective recent research demonstrated that specifcally CerS6 mediated C16 ceramide plays a key role in the development of obesity mediated insulin resistance.

Here we evaluated the therapeutic validity of CerS6 using CerS6 anti-sense oligonucleotide (ASO) to knock-down CerS6 in DIO, db/db and ob/ob mice. CerS6 ASO treatment led to selective and significant ~80% knock-down of the CerS6 expression in the liver and correlated with normalization of plasma C16 ceramide levels compared to control ASO-treated animals. CerS6 knockdown protected in all animal models against body weight gain and was associated with significant reduction in whole body fat content and fed/fasted blood glucose levels. Moreover, insulin resistance was significantly ameliorated as evidenced by significant improvement of oral glucose tolerance tests.

Thus CerS6 dependently generated C16 ceramide represents a distinct sphingolipid species, which contributes to the development of obesity and insulin resistance and therefore represents a unique and attractive novel target to treat obesity and type 2 diabetes.

Title: "hnRNP A2/B1 as a novel post-transcriptional regulator of insulin expression in β-cells"

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Glucose stimulation of β-cells rapidly increases insulin biosynthesis and secretory granule (SG) biogenesis. This increase occurs without affecting the levels of mRNAs coding SG proteins, pointing to post-transcriptional regulation. Several RNA-binding proteins (RBP) that regulate the stability and translation of these transcripts are known, but a comprehensive view of their binding in resting and stimulated conditions is still missing. In this study, cytosolic extracts from rested and glucose stimulated MIN6 cells were used for *in vitro* RNA pull-downs with the biotinylated 5'-UTRs of *Ins1, Ins2*, spliced *Ins2*, *PC2* and *ICA512* mRNAs, and the isolated proteins were identified by mass spectrometry. *γ-tubulin* mRNA 5'-UTR was used as a control for unspecific binging. Using this method, we identified several novel RBPs that interact with mRNAs coding for SG proteins in β-cells. These mRNAs are bound to one set of these RBPs in resting conditions and to a different set of RBPs upon glucose stimulation. One of the novel RBPs, hnRNP A2/B1, was shown to be post-transcriptionally upregulated by glucose, which induced its translocation into the nucleus. hnRNP A2/B1 binding to the 5'-UTR of *mIns1* mRNA was confirmed, and its knock-out in MIN6 cells reduced the levels of *Ins1* mRNA, proinsulin and insulin. Our results indicate that hnRNP A2/B1 regulates the stability of *Ins1* mRNA. To our knowledge, this is the first unbiased proteomics study for the characterization of glucose-induced changes in RBPs associated with mRNAs for SG cargoes. Taken together, our data provide further insight into the mechanisms of glucose-induced insulin biosynthesis and SG biogenesis.

β-Cell primary cilia regulate glucose homeostasis via endosomal EphA-processing

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Primary cilia have recently been implicated in the regulation of glucose metabolism, insulin secretion and islet integrity. However, the precise role of cilia in adult β-cells remains unclear. To address this question, we generated a β-cell specific, inducible cilia knockout mouse. Twelve weeks after induction, glucose homeostasis and insulin secretion deteriorate leading to severe glucose intolerance. We find that loss of cilia and basal body function leads to EphA receptor hyper-phosphorylation and subsequently blocks glucose stimulated insulin secretion. EphA internalization is lost in Ift88-depleted β-cells, partially due to irregular activation of Tiam1-Rac1 signaling and subsequent failure to remodel the actin cytoskeleton. Treatment with NSC23766, a Tiam1-Rac1 inhibitor, restores insulin secretion and EphA/ EphrinA5 internalization. Importantly, we disrupted IFT88 expression in islet preparations from four different organ donors and found elevated EPHA3 phosphorylation as well as impaired insulin secretion in good agreement with our observation in βICKO mice. In conclusion, β-cell primary cilia play a novel role in actin remodeling and endocytosis. When cilia and basal body function is lost, EphA-dependent signaling is activated and ultimately impairs β-cell function, insulin secretion and glucose tolerance in mice and humans.

Marleen Wagner, Stefan Kabisch, Andreas F.H. Pfeiffer

Plasma calculation from full blood glucose levels is not a suitable substitute for direct plasma glucose measurement

Background: The assessment of blood glucose levels is crucial for diagnostic and therapeutic decision in the entire field of diabetologia, including clinical practice and research. As gold standard, diagnostic criteria for diabetes classification are based on plasma glucose levels, despite existing alternative classifications for venous or capillary full blood. Analysing plasma glucose requires centrifugation of the blood sample, which in some cases is not possible. In many situations, such as regular clinical practice, routine diagnostics and a wide range of scientific tests, plasma glucose is exclusively used, as is the most precise way of measurement and time issues are not opposing this decision – it is acceptable to gain results after hours or days. In some occasions, including home testing devices for patients and specific scientific tests such as clamp technique, centrifugation is not available or immediate results are needed for further decisions. Therefore, full blood samples are the only way to assess circulation glucose levels. For these cases, a standard transformation factor of 1,11 (according to Ødum et al., 1999) has been established to calculate plasma values from venous full blood values. It is unclear, how valid and reliable this transformation really is.

Aim: We assess true transformation factors for a large cohort of subjects, undergoing an oGTT and receiving both plasma and full blood glucose measurement, and investigate potential confounding factors.

Methods: We evaluated 342 subjects (71 % women; aged 22-75 years) of a lifestyle intervention study, who underwent a screening oGTT at the clinical ward in Berlin. Glucose levels were assessed both in plasma (NaF) and in full blood (also NaF). Full blood analysis was conducted immediately after blood withdrawal in an EKF Biosen analyzer with regular calibration and 2-monthly interlaboratory tests. Plasma glucose analysis was conducted at the central laboratory with a delay time of 2-4 hours. By division of plasma by full blood values, the true transformation factor (TTF) for each subject and each time point was calculated. Hemoglobin A1c, total hemoglobin, hematocrit and red blood cell count were assessed as correlation parameters. Subjects with anaemia were excluded.

Results: Overall, the average (TTF) for all subjects and all time points was 1,115, with a total range of 0,548 to 2,232. Women had a significantly lower TTF of 1,102 compared to men (1,129). In both sexes, the TTF was significantly lower in fasting state (1,076 and 1,084) compared to postprandial state (t=30'; 1,112 and 1,156). At all time points of the oGTT, TTF was highly correlated with total hemoglobin, hematocrit and red blood cell count, but not HbA1c.

Conclusions: In average, the TTF is close to the fixed factor of 1,11, but wide statistical range, sex-dependency and interaction with fasted state are strong arguments against the use of plasma calculation from full blood samples. As immediate analysis of blood samples with high precision is mandatory for certain scientific techniques (e.g. clamp tests), individual transformation factors based on sex and real-time hematocrit should be established.