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ADIPOSE TISSUE, THERMOGENIC AND METABOLIC ORGAN

28. Identification of the brown and brite adipocyte signature in mice and men

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Recruitment and activation of classical brown and inducible brite/beige adipocytes has received increasing attention in recent years as a strategy to improve systemic metabolic control. Nevertheless, the origin and expression signatures of brown and brite/beige adipocytes are still under debate, mainly due to the complexity of tissue biopsies. To study different adipocyte types in detail, we generated pure samples of brown, brite/beige, and white mature adipocytes by fluorescence activated cell sorting. Employing a machine learning approach for paired analysis of transcriptional data of pure mouse brown, brite/beige and white adipocytes and human brown and white whole adipose tissue obtained by PET-CT-guided biopsies, we were concomitantly able to identify a gene signature that can classify brown and white adipose tissue depots both in rodents as well as in humans. Thus, using the newly developed algorithm, we were able to predict the brown adipocyte content in a mixed population of adipocytes from different human biopsies that can be used for in-depth characterization of complex tissue samples from adipose tissue and might therefore support the development strategies to increase brown adipocyte formation in humans.

29. Metabolic and thermogenic activity of brown fat and obesity–related metabolic disease in men

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There are two major types of adipose tissue in mammals, white and brown. While white adipose tissue provides energy storage/mobilization compartment, brown adipose tissue contains cells specialized to turn chemical energy to heat by uncoupling proton gradient at the inner mitochondrial membrane from ATP production or by utilizing other futile cycling mechanisms. These mechanisms are always physiologically important and under some circumstances they could become energetically very inefficient. Brown fat cells of small mammals use uncoupling protein 1 (thermogenin) to protect from cold. However, alternative UCP1-independent mechanisms of heat production could operate in parallel to boost the acute cold exposure-induced response, or to modulate energy metabolism in response to energy intake. These mechanisms seem to play an important role in human physiology. It is important to note that brown or brown-like adipocytes, with the potential thermogenic capacity in humans are interspersed (possibly generating a functional net) within the specific (perivascular) white adipose tissue depots. Cold-induced activation of brown adipose tissue effectively increases energy expenditure and improves glucose metabolism, directly targeting the basic pathophysiological component of obesity and type 2 diabetes development. Molecular mechanisms of brown fat metabolic/thermogenic activation and their physiological significance in humans are being extensively studied. This research is expected to provide clinically relevant tool to increase energy expenditure, enhance response of our body to exercise and dietary lifestyle changes and minimize thus obesity and associated health risks.

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30. Identification of novel targets with potential to promote brown adipocyte function

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Prevalence of obesity and metabolic syndrome is increasing worldwide and is reaching pandemic proportions in developed countries. This is mainly due to consumption of high-calorie food in combination with sedentary lifestyle. Activation of brown adipose tissue has received a lot of attention in recent years as a promising strategy to increase energy expenditure and improve systemic metabolic control. Several promising molecules with potential to promote brown adipocyte function and white adipocyte browning were identified to date. However, none of them was shown to be effective and produce significant health benefits in clinical trials. Therefore, it is important to search for alternative mechanisms of brown fat activation in adult humans. The aim of our study was to identify novel target genes and mechanisms with potential to promote brown adipocyte function and formation. We collected and analyzed transcriptome and proteome of paired deep neck brown and subcutaneous white adipose tissue samples from 10 patients undergoing neck surgery, as well as transcriptome of PET/CT-guided biopsies of supraclavicular brown adipose tissue and adjacent subcutaneous white adipose of 7 healthy young volunteers. We also analyzed human multipotent adipose derived stem (hMADS) cells differentiated into white and brown adipocytes. By cross-analyzing transcriptomes of whole adipose tissue biopsies and *in vitro* differentiated adipocytes, we were able to identify 742 genes, which are differentially regulated between brown and white adipose tissue/adipocytes, 118 of them by more than 2-fold ($P < 0.05$). Interestingly, UCP1, the main functional effector of uncoupled respiration, was the most highly enriched transcript in brown adipose tissue (437-fold); followed by HMGCS2 (391-fold), the rate-limiting enzyme of ketogenesis; and mitochondrial creatine kinases CKMT1A (188-fold) and CKMT1B (160-fold). In addition, the differentially expressed genes were mainly related to mitochondrial translation, fatty acid metabolism and cellular respiration, based on GO enrichment analysis. Significant differences between brown and white adipose tissue/adipocytes were detected also at the level of proteome. Majority of the 318 proteins with increased abundance in brown adipose tissue was associated with mitochondrial metabolism and confirm the increased oxidative capacity of brown fat cells. In addition to UCP1, we also detected the mitochondrial creatine kinases (CKMT1A/B, CKMT2); as effective modulators of coupled respiration, to be exclusively expressed in brown fat. Our in-depth analysis of human brown and white adipose tissue transcriptome and proteome identified several interesting candidates. Understanding their role in regulation of adipocyte physiology might support development of novel strategies to increase brown fat activity and energy expenditure.

31. Dietary n-3 fatty acids as phospholipids improve insulin sensitivity of the liver and skeletal muscle in dietary obese mice

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Introduction: Nutrition could aid in the prevention of obesity and associated metabolic comorbidities. Previous studies in rodent models of obesity indicated that dietary n-3 fatty acids (Omega-3), namely EPA and DHA, could exert beneficial effects on metabolism, while their phospholipid (PL) form might be more effective than fish oil, i.e. triacylglycerols (TG). Here, we compared the TG and PL form regarding their effects on glucose homeostasis in obese mice. **Methods:** Male C57BL/6N mice (n = 7–10) were fed for 8 weeks a corn oil-based high-fat diet (lipids ~32 wt %; cHF) supplemented with the Omega-3 concentrate either as TG (Epax 1,050 TG from Epax AS; Omega-3 TG) or PL (Krill oil from Olympic Seafood AS; Omega-3 PL) at a dose ~30 g EPA+DHA per kg diet. Glucose homeostasis was assessed by means of glucose tolerance tests and hyperinsulinemic-euglycemic clamps using D-[3–3H]glucose as a tracer. Comparisons were judged to be significant at $p \leq 0.05$ (t-test). **Results:** Compared to cHF, both Omega-3 TG and Omega-3 PL reduced body weight by 8 and 26 %, and hepatic steatosis by 30 and 64 %, respectively. However, only Omega-3 PL reduced fasting blood glucose and plasma insulin by 18 and 59 %, while improving glucose tolerance, i.e. reducing AUC by 38 %. Clamp studies showed elevations of glucose infusion rate, glucose turnover, as well as whole-body glycolysis and glycogen synthesis by 303, 198, 177, and 299 %, respectively, and reductions in hepatic glucose production by 54 %, in the Omega-3 PL group. In contrast, Omega-3 TG only increased whole-body glycogen synthesis by 172 %. Moreover, Omega-3 PL increased the rate of glycogen synthesis in quadriceps muscle by 286 %. **Conclusion:** Our data confirm the superior efficacy of the PL form of Omega-3 regarding the effects on hepatic steatosis and glucose homeostasis, and provide a rationale for the preferential use of Omega-3 PL in clinical practice.

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32. Noninvasive MRI and MRS based approaches to study metabolism in obesity

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Assessment of the tissue metabolism gained a new opportunity with advent and dissemination of the in vivo magnetic resonance spectroscopy (MRS) and imaging (MRI) methods. Skeletal muscle, liver, myocardium and fat tissue are easily accessible for investigation. MRI allows for the assessment macroscopic fat distribution, while MRS applications allows for the quantification of ectopic of fat compartments and their saturation profile. Characterization of glucose fluxes from biopsy specimen could have been replaced by ^{13}C and ^{31}P MRS, which was able to quantify defects of glucose metabolism in both skeletal muscle and liver in diabetes and other insulin resistant states. Basal and stimulated intracellular energy metabolism can be monitored by ^{31}P MRS and combination of spectroscopy and imaging examinations of the skeletal muscle function and energetic metabolism can help to identify the links between impaired metabolism and function. Selected issues with respect specific organs, and research as well as clinical applications will be presented and discussed.

33. Treatment of osteoarthritis with freshly isolated stromal vascular fraction cells from adipose and connective tissue

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Degenerative osteoarthritis affects more than 50 % of people older than 50 years. People with obesity or overweight suffer most frequently. Therapy of osteoarthritis relies on non-steroid analgesics, chondroprotectives and in late stages total joint replacement is considered a standard of care. We performed a pilot study using novel stem cell therapy approach that was performed during one surgical procedure. It relies on abdominal lipoaspiration and processing of connective tissue to stromal vascular fraction (SVF) cells that typically contain relatively large amounts of mesenchymal stromal and stem cells. SVF cells are injected immediately to the target joint or to the connective tissue of the target joint. Since 2011, total of 1,128 patients have been recruited and followed for up to 42 months to demonstrate the therapeutical potential of freshly isolated SVF cells. At the same time, one to four joints (knees and hips) were injected with SVF cells per patient. A total number of 1,856 joints were treated. Clinical scale evaluation including pain, non-steroid analgesic usage, limping, extent of joint movement and stiffness was used as measurement of the clinical effect. All patients were diagnosed with stage II–IV osteoarthritis using clinical examination and X-ray, in some cases MRI was also performed to monitor the changes before and after stem cell therapy. After 12 months from SVF therapy, at least 50 % clinical improvement was recognized in 91 %, and at least 75 % clinical improvement in 63 % of patients, respectively. Within 1–2 weeks from SVF therapy 72 % of patients were off the non-steroid analgesics and most of them remain such for at least 12 months. No serious side effects, infection or cancer was associated with SVF cell therapy. In conclusion, here we report a novel and promising therapeutical approach that is safe, cost effective, and relying only on autologous cells.

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