Poster #1

In PDE3B KO Mice, White Epididymal Adipose Tissue (WAT) Exhibits Phenotypic Characteristics of "Fat-burning" Brown Adipose Tissue (BAT), with Reduced Expression of Pro-inflammatory Markers including the NLRP3 Inflammasome

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Obesity is a major risk factor for cardiovascular diseases and type 2 diabetes. In white epididymal adipose tissue (WAT) of PDE3B KO mice in SvJ129 background, cAMP/PKA- and AMP-activated protein kinase (AMPK)-signaling pathways are activated, the integration of which results in KO WAT assuming phenotypic characteristics of brown adipose tissue (BAT), including alterations in morphology and increased expression of genes, such as PGC-1α, PDRM16, LRP130, and Elovl3, that are important in mitochondrial biogenesis, induction of the thermogenic program, and differentiation/recruitment of BAT. In KO WAT, there is coordinate regulation of expression of genes, transcriptional regulators, and mitochondrial proteins required for energy dissipation and fatty acid oxidation, such as PPARα, ATGL, perilipin, UCP-1, CIDEA, and several mitochondrial proteins involved in election transport and fatty acid β-oxidation. UCP-1, a "marker' for brown adipose tissue (BAT) usually not present in WAT, is markedly elevated in KO WAT. These findings contribute to several phenotypic characteristics of PDE3B KO mice, including a smaller increase in body weight in response to high fat diets, smaller gonadal fat deposits and adipocytes, uncoupled EWAT mitochondrial respiration, increased oxygen consumption, increased fatty acid oxidation in PDE3B KO adipocytes, and increased treadmill endurance. In addition, expression of a number of inflammation-related genes was decreased in KO WAT, including some whose reduced expression might reflect reduced monocyte/macrophage infiltration/differentiation in KO EWAT as well as a reduction in the NLRP3 inflammasome. These results suggest that PDE3B may regulate a cAMP-sensitive "switch" for WAT/BAT phenotypic conversion, regulating downstream effects of cAMP on cAMP/PKA- and AMPK-signaling, mitochondrial biogenesis and function, energy dissipation, and the pro-inflammatory state of WAT. Understanding mechanisms for these changes in KO EWAT is important, since conversion of "fat-storing" EWAT to "fat-burning" BAT, with reduced inflammation, represents a potential strategy in treatment of obesity and diabetes.

Poster #2

Brown Adipose Tissue and Aerobic Performance of Athletes

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Relationship between brown adipose tissue (BAT) in humans and cold exposure have been studied during last 60 years (see Lee, Swarbrick, & Ho, 2013; van Marken Lichtenbelt, 2012). Recent findings have demonstrated a close relationship between BAT and physical activity as well (Boström et al., 2012; Spiegelman, 2013). Longitudinal studies of athletes with different levels of physical fitness (including use of thermography) have discovered some new facts. In particular, there exists a relationship between the maximal aerobic capacity and BAT activity in moderate cold exposure at rest.

Methods: The study involved 55 male athletes (23.5 ± 4.9 yr, weight 70 ± 11.5 kg, height 174 ± 7 cm) from different sports with different levels of physical fitness. All participants underwent medical tests confirming that their functional status does not prevent participation in the experiment, and written informed consent was obtained from each participant. First stage included non-shivering activation of thermogenesis (room temperature 20-21 ° C, humidity 45-50%). Registration of skin temperature at the back and neck areas has been made through infrared thermography (NEC TH 9100SL). Moderate cold exposure time was 15 minutes. Subjects kept position without movement and were stripped to the waist. To analyze BAT activity, we measured weighted average (WAT), the minimal (MNT) and maximal (MXT) skin temperature of the back and neck. Then all participants performed ramp test on a treadmill with measured gas exchange (Oxycon Pro) and the concentration of blood lactate (Biosen C_line). Lactate was measured every 2 minutes during the ramp-test and 10 minutes after tests cessation. Performance indicators were maximal oxygen consumption (VO2max), anaerobic threshold (AT), and the blood lactate concentration.

Results: Skin temperature decreased from baseline after 15 minutes of non-shivering thermogenesis with naked upper body without movements. Skin temperature after adaptation was on average: MXT 33.7 ± 0.49 ° C; WAT 31.7 ± 0.54 ° C; MNT 29.0 ± 1.13 ° C. Correlation analysis revealed a positive relationship between indicators of aerobic performance and thermograms in muscular rest, reflecting the activity of BAT. Amongst three indicators of the thermogram (WAT, MNT, MXT), best relation with the parameters of aerobic performance indicators had WAT and MNT (Spearman correlation coefficients between the AT and WAT: 0.72; VO2max and WAT: 0.61, VO2max and MNT: 0.68, AT and MNT: 0,69 ($p < 0.05$)). These data suggest that aerobic performance and non-shivering thermogenesis in a slight activation of thermogenesis are related with each other. Lactate concentration was also correlated (negatively) with skin temperature, but only in the recovery period after exercise. We found a correlation between the maximum concentration of blood lactate (which is usually detected by 3-5 minutes after exercise)

and the WAT ($r=-0.68$, $p<0.05$). This is consistent with some previous findings (De Matteis et al., 2012). Particularly, physical exercise was reported to improve the activity of BAT, and activate the transport of the MCT1 protein (specific lactate transporter) across the cell membrane at the same time. Our data (Son'kin et al., 2010, 2011) as well as contributions from other authors (Slocum et al., 2012; Villarroya, 2012) provide evidence to conclude that brown adipose tissue plays an important role in the maintaining homeostasis during vigorous exercise and recovery.

Poster #3

In Vivo Remodeling of White Adipose Tissue into Brown-like Adipose Tissue, via a Novel Small-Molecule Drug-Candidate

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White-adipose-tissue (WAT) to brown-like adipose-tissue (BAT) conversion is considered a leading strategy to combat obesity. Currently, most efforts are directed towards genetic manipulation of individual adipocytes or preadipocytes, mainly in rodents.

We have serendipitously discovered that intra-fat injection of a synthetic small-molecule (RZL-017, MW: ~450 Da) can induce sequential remodeling of WAT into BAT in mice, rats and pigs. In pigs, RZL-017 was injected into the subcutaneous fat, a model system suitable for treatment of human obesity.

Following injection of RZL-017 into WAT, massive fat necrosis was observed in all treated animals, with no sign of inflammation. Consequently, the tissue morphology changed, with an increased number of blood vessels and appearance of "foamy" BAT-like adipocytes. Monitoring of treated animals by infra-red imaging, demonstrated local elevation of temperature that lasted for at least 2months, confirming the formation of thermogenic tissue around the injected site. At the molecular level, WAT-to-BAT phenotypic transformation was accompanied by a ~50-fold increase in UCP1 gene-expression in mouse epididymal fat. In-vitro, RZL-017 was shown to inhibit NIH-3T3-L1 preadipocyte differentiation into mature adipocytes and to enhance PGC1 expression by about 6-fold.

Finally, RZL-017 intra-WAT injection triggered a significant reduction in adipose-tissue mass in all treated animals.

Note: The chemical structure of RZL-017 and its putative molecular target will only be disclosed at a later stage, due to intellectual property considerations.

Financial interest disclosure: *M. B-S is a co-founder of Raziel Therapeutics Ltd. which holds the license for RZL-017 commercialization.

Poster #4

Acute Cold Exposure Elicits Increases in BAT Oxidative Metabolism in Individuals with Type 2 Diabetes*

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We recently showed that BAT can significantly contribute to cold-induced thermogenesis and that a very small proportion of this heat production is fueled by circulating fatty acids and glucose. We and others have also demonstrated that the prevalence of spontaneously activated BAT is lower in diabetic compared to non-diabetic patients. However, the oxidative capacity and substrate utilization of cold-activated BAT in type 2 diabetes (T2D) has never been examined. The objective of this study was to quantify oxidative metabolism and glucose and fatty acid uptake of BAT during a mild-cold exposure in men with T2D. BAT volume of activity, glucose and NEFA uptake and oxidative metabolism were determined using positron emission tomography coupled with computed tomography (PET/CT) with [18F]fluorodeoxyglucose (18FDG), 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid(18FTHA) and [11C]acetate in healthy control subjects and individuals with T2D exposed to mild-cold. Shivering intensity was examined using surface electromyography (% of maximal voluntary contraction) of eight major muscles. Our results show that men with type 2 diabetes examined to date demonstrate a similar increase in cold-induced thermogenesis, measured using indirect calorimetry, and cold-induced BAT oxidative metabolism, despite smaller volumes of metabolically active BAT compared to healthy men (BAT volume of 6 ± 2mL vs. 59 ± 15 mL). Rather, shivering intensity was shown to be nearly double in men with T2D compared to the healthy control men (4.6 ±1.0 vs. 2.4 ± 0.5 % of maximal voluntary contraction, P= 0.09). In conclusion, the volume of metabolically active BAT appears smaller in T2D but is still metabolically active. The influence of the many co-factors associated with T2D, including age, adiposity or other metabolic and endocrine factors may be responsible for these differences but this remains to be investigated.

*Abstract chosen for a Hot Topic talk

Poster #5

Brown Adipose Tissue Activity and Thyroid Hormone in Adult Humans

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Background: Cold-stimulated non-shivering thermogenesis in brown adipose tissue (BAT) is suggested as a possible therapeutic target in the prevention and treatment of obesity in rodents. Thyroid hormones have a direct influence on brown adipocyte sensitivity to the sympathetic nervous system and stimulate of the hypothalamus, thereby activating the sympathetic pathway. The primary objective of this study is to examine the effect of thyroid hormone on brown adipose tissue activity and non-shivering thermogenesis in adult humans.

Methods: Study design BAT activity was measured twice in thyroid gland cancer patients. These measurements took place during the standard treatment protocol of well-differentiated thyroid cancer, including total thyroidectomy and subsequent radioactive iodine ablation. The first measurement was carried out after total thyroidectomy, in which serum thyroid hormone (thyroxin) levels were low or non-existing and serum thyroid stimulating hormone (TSH) was consequently high (hypothyroid phase). The second measurement took place after radioactive iodine ablation and TSH-suppressive doses of levothyroxine, administered to the patients until a euthyroid near-to-hyperthyroid hormone level status was reached. In this second phase thyroxine levels were high and TSH levels low (euthyroid phase).

Protocol: ^{18}F-FDG PET-CT-imaging was performed under mild cold stimulation to assess BAT activity during the two conditions. In order to achieve maximum non-shivering thermogenesis and BAT activation an individualized cooling protocol was used. Energy expenditure was measured with a ventilated hood system.

Preliminary Results: Preliminary analysis of the data of 5 subjects (1 male and 4 female) showed that basal metabolic rate (BMR) was 20% higher in the presence of thyroid hormone (BMR 4.23 ± 0.11 kJ/min versus 3.53 ± 0.50 kJ/min). BAT activity (SUV_{mean}) increased in 4 patients (+15%, +42%, +20%, +26%), and decreased in one patient (-16%). Overall the data indicated an increase in BAT activity (BAT Mean SUV_{Mean} 3.3 in euthyroid phase versus 2.7 in hypothyroid phase) and a 35% increase in non-shivering thermogenesis (NST 0.62 ± 0.22 kJ/min versus 0.46 ± 0.39 kJ/min) in the presence of high levels of thyroid hormones, compared to the situation with low levels of thyroid hormone after thyroidectomy.

Conclusion: The preliminary results show a positive effect of thyroid hormones on brown adipose tissue activity and energy expenditure in humans. Thyroid dysfunction seems to negatively affect brown adipose tissue activity. New targets for the prevention and treatment of obesity may need to take this BAT-thyroid interaction into account.

Poster #6

Characterization of the Nature, Thermogenic Properties and Inflammatory Profile of Human Epicardial and Mediastinal Fat Depots

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Human heart is surrounded by multiple fat depots, which are commonly referred to as the pericardial adipose tissue. Fat around the heart, however, can be anatomically distinguished into epicardial adipose tissue (eAT) and paracardial- or mediastinal- adipose tissue (mAT). In humans, eAT represents the innermost layer of visceral pericardium, whereas mAT sits outside the pericardium. Due to its close proximity to myocardium and its ability to share common blood supply with it, eAT has been proposed to play an important role in maintaining heart function. In accordance, numerous studies have observed negative associations between eAT mass and volume, its oxidative stress levels and inflammatory profile with onset and progression of coronary heart disease in humans. Literature regarding the associations between accumulation of mAT and heart disease also points in a similar direction. However, it must be acknowledged that our understanding of fat depots around the heart is skewed by studies that do not distinguish various fat depots and usually refer to them as a common fat depot. We have previously observed significantly higher expression of UCP1 in eAT relative to mAT in humans. In an attempt to characterize the thermogenic properties of the individual fat depots around heart and to clarify their physiological roles, we investigated the beige related-, thermogenic- and inflammatory-markers in human eAT and mAT. Biopsies of eAT, mAT and subcutaneous adipose tissue (sAT) were obtained from patients (n=33) undergoing coronary artery bypass grafting procedure at *Quebec Heart and Lung Institute*. Gene expression data of eAT and mAT were normalized to sAT and analyzed using paired t-tests. We observed higher expression of beige-specific markers PRDM16, TBX1 and TMEM26, and lower expression of classic brown fat-specific markers EVA1 and LHX8 in eAT relative to mAT in our cohort. Human eAT also exhibited higher expression of LPL, COX4, VLCAD, LCAD, MCAD and SCAD relative to mAT. Additionally, eAT displayed higher expression of pro-inflammatory genes IL6, TNFα and MCP1, as well as that of the anti-inflammatory genes adiponectin, natriuretic peptide receptor C and adrenomedullin, relative to mAT. While no direct associations were observed for beige- and most inflammatory-markers, eAT expression of adiponectin, UCP1 and COX4 associated negatively with circulating TG levels. Additionally, eAT expression of LPL, VLCAD, LCAD, MCAD and SCAD associated with higher circulating HDL cholesterol and lower TG levels.

On the contrary, no such associations were observed for mAT expression of these genes. In conclusion, eAT exhibited a stronger beige phenotype, higher thermogenic properties and mixed inflammatory profile relative to mAT in our cohort. Exclusive associations between eAT and circulating lipids may further point towards a more metabolically active role for eAT than mAT in humans.

Poster #7

Use of Positron Emission Tomography (PET) Tracers to Assess Brown Adipose Tissue (BAT) Metabolism in Cold-adapted, Cold-exposed Rats

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BAT represents an exceptional heat-producing and energy-burning tissue. BAT has been postulated to play a pivotal role in thermoregulatory thermogenesis and energy balance regulation. Cold exposure was demonstrated to be one of the most powerful BAT activator through the sympathetic nervous system (SNS). The present study was designed to investigate BAT metabolism during cold exposure after cold adaptation, using PET tracers.

Male Wistar rats were housed for three weeks either in a thermoneutral (27°C) or in a cold (10°C) environment. After the adaptation period, rats were exposed to 10°C for 2 and 6 hours. Energy substrate metabolism was assessed using positron emission tomography with ^{11}C-Acetate, ^{18}F-fluorodeoxyglucose (FDG) and ^{18}F-fluoro-thia-heptadecanoic acid (FTHA).

Our results show that cold adaptation, compared to warm adaptation, was associated with an increase in food intake without weight gain (0.14 *vs.* 0.25 g of weight gain per gram of food consumed). Warm adaptation completely inactivated BAT capacity and metabolism. Six hours of cold exposure were sufficient to increase oxidative metabolism and glucose/NEFA uptake in BAT of warm-adapted rats. Additionally, we found that cold adaptation increased BAT blood flow and BAT oxidative metabolism as measured with ^{11}C-Acetate. It is noteworthy that the oxidative metabolism index was 1.5-fold higher in BAT than in heart. Indirectly, we have been able to measure the possible contribution of circulating TG in BAT, which seems to be very important during cold exposure.

We conclude that cold adaptation increases the oxidative capacity of BAT and that cold exposure, which stimulates SNS-mediated BAT thermogenesis, represents an effective condition to increase energy expenditure through BAT and to diminish energy gain. Moreover, our study reveals the benefit and practicality of using PET tracers to assess the metabolism of BAT during acute and chronic cold exposure.

Poster #8

Brown Adipose Tissue Improves Glucose Metabolism and Whole Body Insulin Sensitivity in Humans*

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Despite the intense scientific interest regarding the existence, plasticity and localization of human brown adipose tissue (BAT), its functional role on whole body metabolism in humans still remains unclear. To address this, we studied males with high (HBAT, n=7) and low (LBAT: n=5) brown adipose tissue volume (69±18 mL vs 4.4±2.3 mL, $p<0.05$) under non-shivering cold exposure (CE) and thermoneutral (TN) conditions using Positron Emission Tomography-Computed Tomography (PET/CT), stable isotope infusion, a hyperinsulinemic euglycemic clamp, and indirect calorimetry. The subjects in the two groups were similar in terms of age, BMI, abdominal visceral and subcutaneous adiposity. During CE, only HBAT subjects demonstrated a significant increase in resting energy expenditure (TN: 0.039±0.003 kcal/kg/min vs CE: 0.045±0.004 kcal/kg/min, $p<0.05$), plasma glucose (TN: 9.7±0.7 µmol/kg/min vs CE: 11.3±0.4 µmol/kg/min, $p<0.05$) and free fatty acid oxidation (TN: 5.9±1.0 µmol/kg/min vs CE: 2.7±0.6 µmol/kg/min, $p<0.05$), whole body glucose uptake under basal (TN: 9.7±0.7 µmol/kg/min vs CE: 11.3±0.4 µmol/kg/min, $p<0.05$) and insulin-stimulated conditions (TN: 13.0±2.1 µmol/kg/min vs CE: 16.5±1.1 µmol/kg/min, $p<0.05$), and insulin sensitivity (TN: $1.5\pm0.3*10^{-3}$ dL/kg/min(µU/mL) vs CE: $2.1\pm0.3*10^{-3}$ dL/kg/min/(µU/mL), $p<0.05$) compared to TN conditions. LBAT subjects did not demonstrate any significant change with CE in the previously mentioned parameters. These findings confirm the functional role of BAT in whole body substrate metabolism and insulin sensitivity and provide the first convincing evidence in humans that BAT activation can combat insulin resistance and diabetes. Further studies will illuminate additional lifestyle or pharmaceutical interventions, which may activate BAT and/or induce the browning of the more abundant white adipose tissue *in vivo*. If the potential of adipocytes to alter energy metabolism and expenditure is full realized, adipose tissue may become a therapeutic target in the battle against obesity and its associated metabolic consequences.

*Abstract chosen for a Hot Topic talk

Poster #9

GPAT4 Regulates the Metabolism of Glucose and Fatty Acid in BAT

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Glycerol 3-phosphate acyltransferase (GPAT) is the initial and rate-limiting enzyme in the synthesis of triacylglycerol (TAG) and catalyzes the addition of a fatty acyl-CoA to the *sn*-1 position of glycerol 3-phosphate. Four mammalian GPAT isoforms exist, each a product of a separate gene. GPAT4 is the major GPAT isoform in BAT, comprising 65% of the total GPAT specific activity. Cultured primary brown adipocytes from *Gpat4*$^{-/-}$ mice incorporated 40% less fatty acid into TAG and oxidized fatty acid at a 46% higher rate than controls. Despite normal fatty acid uptake into brown adipose tissue (BAT) of *Gpat4*$^{-/-}$ mice fed a high-fat diet, BAT TAG content was 24% lower than in controls. Furthermore, expression of *mCpt1*α, *Pgc1*α, and *Ucp1* in BAT of *Gpat4*$^{-/-}$ mice fed a high-fat diet was 8, 5, and 2.5-fold higher, respectively. Consistent with the dietary-induced changes in gene expression, mitochondria isolated from BAT of *Gpat4*$^{-/-}$ mice fed a high-fat diet oxidized 200% more oleate and 150% more pyruvate to CO_2 than in controls. These data suggest that in the absence of GPAT4 in BAT, cellular metabolism shifts to promote uncoupled mitochondrial oxidation and thermogenesis.

Poster #10

Passive Microwave Radiometry for the Non-Invasive Detection of Human Brown Fat: First Human Studies with Direct Correlation to FDG/PET

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Background: Passive microwave radiometry is a method of detecting the inner temperature of an object via the intensity of electromagnetic radiation present at its surface. Originally designed for use in areas such as astronomy and geophysical exploration, it has been shown previously to have potential use in the clinical setting as a noninvasive tool for the detection of malignancy or enhanced metabolic activity. In contrast to imaging modalities such as PET/CT, this method is inexpensive , does not involve ionizing radiation exposure, and does not involve injection of a substance into the subject.

Human neoplasms are associated with an increase in localized metabolism, which corresponds to an increase in heat generated within those tissues. Recent findings suggest that brown adipose tissue (BAT) may be a suitable research model comparable to tumors. BAT is one of two types of fat found in humans. Contrary to white adipose tissue (WAT), which is used for energy-storage, the function of BAT is to dissipate energy in the form of heat. BAT has high glucose uptake when activated and is visible using fluoro-deoxy-glucose (FDG) PET/CT. This uptake can be modulated via changes in ambient temperature or through the use of beta blockers.

The purpose of this study was to evaluate the operating characteristics of the microwave radiometer in the assessment of human BAT activity in stimulated and suppressed conditions, and compare this to the imaging gold standard, FDG PET/CT.

Materials and Methods: Nineteen participants were scanned with FDG PET/CT and the radiometry system using a cold intervention to activate brown fat. During the cold condition participants had their feet on a cold block of ice for 4 out of every 5 minutes for 1 hour prior to FDG injection and for 2 hours prior to PET/CT imaging. They were also in a cooled room ($17^{o}C$ – $21^{o}C$) during the ice exposure. Eleven of these participants exhibited brown fat activity qualitatively on PET and were scanned again, about two weeks later, with both methods following a protocol designed to minimize activated BAT. During the warm, BAT minimization conditions, participants were seated in a warm room ($24^{o}C$ – $28^{o}C$) and given a 20 mg dose of propranolol 2 hours prior to FDG PET/CT imaging.

Radiometry was performed for two hours prior to PET imaging during both warm and cold interventions. A grid of 15 – 20 points was drawn on the participant's upper body and a photo was taken for comparison with PET images. Beginning every 5 minutes, radiometry

data was collected by placing the radiometry probe on each point until the signal stabilized (about 3 seconds). The researcher responsible for placing the probe was blinded to the recorded radiometer measurement.

FDG was injected approximately one hour prior to PET imaging. Whole body PET scans were performed from thigh to top of the head.

Results: PET identified brown fat activity in 11 of 19 participants. In 9 of these 11 participants, radiometry measurements collected during the cold study were modestly, but significantly higher on points located over areas of active brown fat on PET, than in points not exhibiting BAT activity ($P < 0.01$). This difference lessened during the warm studies: 7 of 11 participants showed radiometry measurements that did not differ significantly between the same set of points. During cold studies, the average radiometer measurement across all points was approximately $3^{o}C$ cooler than measurements taken during warm studies.

Conclusion: Passive microwave radiometry was shown to be feasible and, with some improvements, has the potential to reliably detect active brown fat using the participant's non-activated brown fat skin area as a control. The radiometry system appears to be quite sensitive to ambient temperature as indicated by the higher average radiometer temperature measurement for participants whose BAT was inactivated on PET by a warm room and a beta blocker. Apparent sensitivity to detecting superficial skin temperature is a probable limitation to the passive radiometry approach used in this study.

Poster #11

Mild Intermittent Cooling of Mice is Sufficient to Increase the Thermogenic Capacity of the Adipose Tissue Depots

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In rodents, chronic, persistent cold exposure at 4 °C increases interscapular brown adipose tissue (BAT) thermogenic capacity and induces brown adipogenesis within white adipose tissue (WAT) depots. In humans, recent studies have shown that milder, intermittent cooling for even ten days increases BAT activity, but the limitations of the clinical studies prevent accurate anatomical, histological, and gene expression profiling of the physiological changes. In this study, we determined how chronic, mild intermittent cooling affects hyperplasia and hypertrophy of the adipose tissue depots. Four different sets (n=6) of 10 week-old C57Bl/6 mice (Jackson Labs) were housed at room temperature (23 °C) and exposed to mild cold (14 °C) for the following periods during their light cycle: 2, 6, or 10 hours. The fifth set remained continuously at 14 °C. After 18 days, at the end of the dark cycle, plasma glucose was measured, and tissue was resected for analysis. There was no difference among the body weights or random fed glucose (both $P<0.05$). However, BAT mass was increased both in absolute terms and in proportion to body weight in mice exposed to 14 °C for both 10 hours and 24 hours each day ($P<0.02$ or lower for all comparisons). At 14 °C, the interscapular BAT (iBAT) had higher levels of UCP1 expression ($P<0.004$), but no change in PPARg ($P>0.05$) indicating hypertrophy but not hyperplasia. The inguinal WAT (ingWAT) changed even more, demonstrating significantly higher UCP1 even in the mouse cooled for only 2 hours each day ($P<0.01$), and new brown adipocytes could be seen in the mice cooled for 10 and 24 hours ($P<0.003$ each). In contrast, the perigonadal WAT (pgWAT) had lower UCP1 with even 2 hours of cooling ($P<0.001$). In summary, we show that mild cooling of mice at 14 °C for only 18 days leads to substantial changes in the adipose tissue depot: there is increased thermogenic capacity in the iBAT; brown adipogenesis in ingWAT; but a suppression of browning in the pgWAT. In addition, intermittent cooling for as little as 2 hours each day is capable of inducing browning of the ingWAT depot. These results suggest that mild intermittent cooling of humans could also induce browning of the analogous adipose tissue depots and permit increased energy expenditure.

Poster #12

The Function of IMP2 in Nutrient Metabolism

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IMP2 is an RNA binding protein and functions in RNA post-transcriptional regulation. Previously, we have shown that IMP2 is an mTOR substrate whose insulin/amino acid stimulated phosphorylation promotes mRNA translation. Recently, an association between SNPs in the second intron of the IMP2 gene and type 2 diabetes mellitus (T2DM) has been detected in human genetic study. However, the underlying physiological functions of IMP2 in metabolism or in the pathogenesis of T2DM remain undefined. Here, we report that IMP2 null mice are lean, protected from diet-induced obesity and insulin resistance, due to increased energy expenditure through brown fat thermogenesis. In mice, the absence of IMP2 increases the abundance of uncoupling protein 1 (UCP1) in brown fat by directly binding UCP1 mRNA and represses its translation. Finally, we show that the mature isolated brown fat cells have increased uncoupled respiration and oxygen consumption. Therefore, our study has provided new opportunities for preventing and/or treating obesity-related T2DM by targeting IMP2.

Poster #13

Characterization of Brown Adipose Tissues using MRI in the Pediatric Population – a Pilot Study

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Background: With the early onset of obesity, children and adolescents are at risk of developing many medical conditions such as hypertension, type 2 diabetes and fatty liver disease. Obesity results from an imbalance between energy intake and expenditure. Recent studies indicate that brown adipose tissue (BAT) is an adaptive thermogenesis organ that dissipates energy directly by heat. Functionally deficient or hyperactive BAT may contribute to development of obesity[1]. Metabolically inactive BAT accumulates intracellular lipid thereby resulting in more white adipose tissue (WAT)-like tissues[2]. Recently, a third kind of adipose tissue consisting of BAT-like cells (known as 'beige' or 'brite' adipocytes) has been discovered, and it can be induced within WAT by cold stimulation[3]. The current 'gold-standard' imaging method to measure BAT uses FDG-PET imaging and has revealed functional BAT in human adults by monitoring the increased glucose uptake under cold exposure; however, the radiation dose largely impedes its application in pediatric population. Alternatively, MRI has increasingly been used for differentiation between BAT and WAT based on the measurement of fat percentage and T2*, which reflect the fact that BAT contains small multilocular lipid droplets and abundant iron-rich mitochondria; whereas WAT contains single large lipid droplets and less cytoplasm. In addition, BAT is characterized by more profuse capillary vascularization with increased blood flow compared to WAT, which may be potentially explored by MRI. MRI studies comparing BAT characteristics in the pediatric population have been limited. In this pilot study, we proposed to exploit chemical-shift and diffusion-weighted MRI methods to characterize brown adipose tissues located at the cervical-supraclavicular areas, and compare the tissue composition between normal-weight and obese pediatric groups.

Methods: This study was approved by our Institutional Review Board. A total of 20 healthy children (9-15 years) enrolled and were divided into four groups: Group1 (G1, N=6): lean/prepubertal, Group2 (G2, N=3): obese/prepubertal, Group3 (G3, N=5): lean/pubertal, and Group4 (G4, N=6): obese/pubertal.
MRI Acquisition: Imaging was performed on a 3.0T MRI scanner (Magnet Trio, Siemens Medical Solutions, Erlangen, Germany) using a 12 channel head/neck coil. The chemical shift imaging protocol includes a 3D T1W VIBE 2-point Dixon sequence for initial neck fat distribution mapping, followed by a 2D six-echo (i.e. 6-point) spoiled gradient echo (SPGR) sequence for quantitative measurement of fat percentage (FP) and T2*. The imaging parameters for 2D 6-point SPGR were: coronal plane, FOV_{read} = 320-360mm^2, number of

slices = 15-20, TR = 250ms, TE = 2.46, 3.69, 4.92, 6.15, 7.38 and 8.61ms, corresponding to consecutive in- and out-of-phase echo time, TH/gap = 4/1mm, matrix = 192×160, average =3, iPAT=2, flip angle=10° to reduce the T1 weighting, saturation band covering the heart to reduce flow/pulsation artifacts. Diffusion-weighted (DW) imaging was performed using the BLADE sequence. DW-BLADE is a multi-shot turbo-spin-echo (TSE) based sequence with inherent motion correction properties and is insensitive to chemical shift and susceptibility artifacts. The imaging parameters were: FOV = 380×380mm^2, number of slices = 15-20, TR = 4500ms, TE = 190ms, TH/gap = 4/1mm, matrix = 192×192, echo train length = 17, average =1, iPAT=3, b-value = 10, 50, 100, 500 and 2000 s/mm^2, no fat saturation.

Post Processing: A stack of initial fat percentage (FP) maps throughout the neck were generated from 3D VIBE 2-point Dixon images for overview and identification of BAT regions (**Fig.1a**). Regions of interest (ROIs) were then drawn in the presumed BAT areas on 2D SPGR images (**Fig.1b**), where the signal intensity oscillation in BAT was observed along TE (**Fig.1c, d**). Fat percentage (FP) and T2* were calculated within each ROI, based on a multi-point, multi-fat-peak model as described in **Fig. 2**. This model also took account of bi-exponential T2* decay (i.e. assuming $T2^*_{water} \neq T2^*_{fat}$). On the basis of 3D VIBE FP maps, BAT ROIs were drawn on DW images (b=10 s/mm^2), where fat appeared as bright signals. The signal decay within each ROI was fitted to calculate the apparent diffusion coefficient (ADC); in addition, pseudo-perfusion parameters including fast diffusion efficient (D_{fast}) and fast diffusion volume (V_{fast}) were calculated based on the equation: $S(b)/S0 = (1- V_{fast})\cdot e^{-bD} + V_{fast}\cdot e^{-b(D+Dfast}$). A Levenberg-Marquardt non-linear fitting algorithm (Matlab software, Mathworks, Natick, MA, USA) was used for all nonlinear model fitting.

Statistical Analysis: For all ROIs drawn in neck BAT areas, FP, T2*, ADC, D_{fast} and V_{fast} of BAT tissues were compared between any pair of the four groups. Multilevel linear regression analyses were performed to assess the statistical difference among the four groups, with $p<0.05$ indicating significant statistical difference.

Results: *FP measurement*: Obese groups (FP_{G2}=60.4±0.89% and FP_{G4}=56.8±5.6%) showed significantly higher FP than lean groups (FP_{G1}=48.3±7.5% and FP_{G3}=46.4±8.3%), whereas FP was not affected by pubertal stage (i.e. G1 *vs.* G3 and G2 *vs.* G4) (**Fig.3a**). *T2* measurement*: The prepubertal lean group ($T2^*_{G1}$=8.9±0.99ms) had significantly lower T2* compared to the other three groups ($T2^*_{G3}$=11.2±2.4, $T2^*_{G2}$=12.9±4.5 and $T2^*_{G4}$=12.0±3.1ms). Higher T2* values in older and obese groups indicated less mitochondria content in their BAT tissues (**Fig.3b**). Over all measured imaging pixels, the scatter plot of joint FP and T2* distribution showed that obese groups tended to have higher FP and T2* in BAT tissues, compared to lean groups (**Fig.4**). *ADC measurement:* The ADC values in BAT were not significantly different between any two groups (ADC_{G1}=0.51±0.09, ADC_{G3}=0.52±0.14, ADC_{G2}=0.54±0.10 and ADC_{G4}=0.48±0.04×10^{-3} mm^2/s) (**Fig.3c**). *Pseudo-perfusion measurement:* Higher D_{fast} and V_{fast} are related to increasing capillary blood flow. There was no significant difference in D_{fast} between any two groups ($D_{fast,G1}$=8.5±3.8, $D_{fast,G3}$=7.9±2.6, $D_{fast,G2}$=9.9±3.1 and $D_{fast,G4}$=10.2±3.1×10^{-3}mm^2/s) (**Fig.3d**). Prepubertal, both lean and obese groups ($V_{fast,G1}$=45.3±8.1%, $V_{fast,G2}$= 46.2±1.3%)

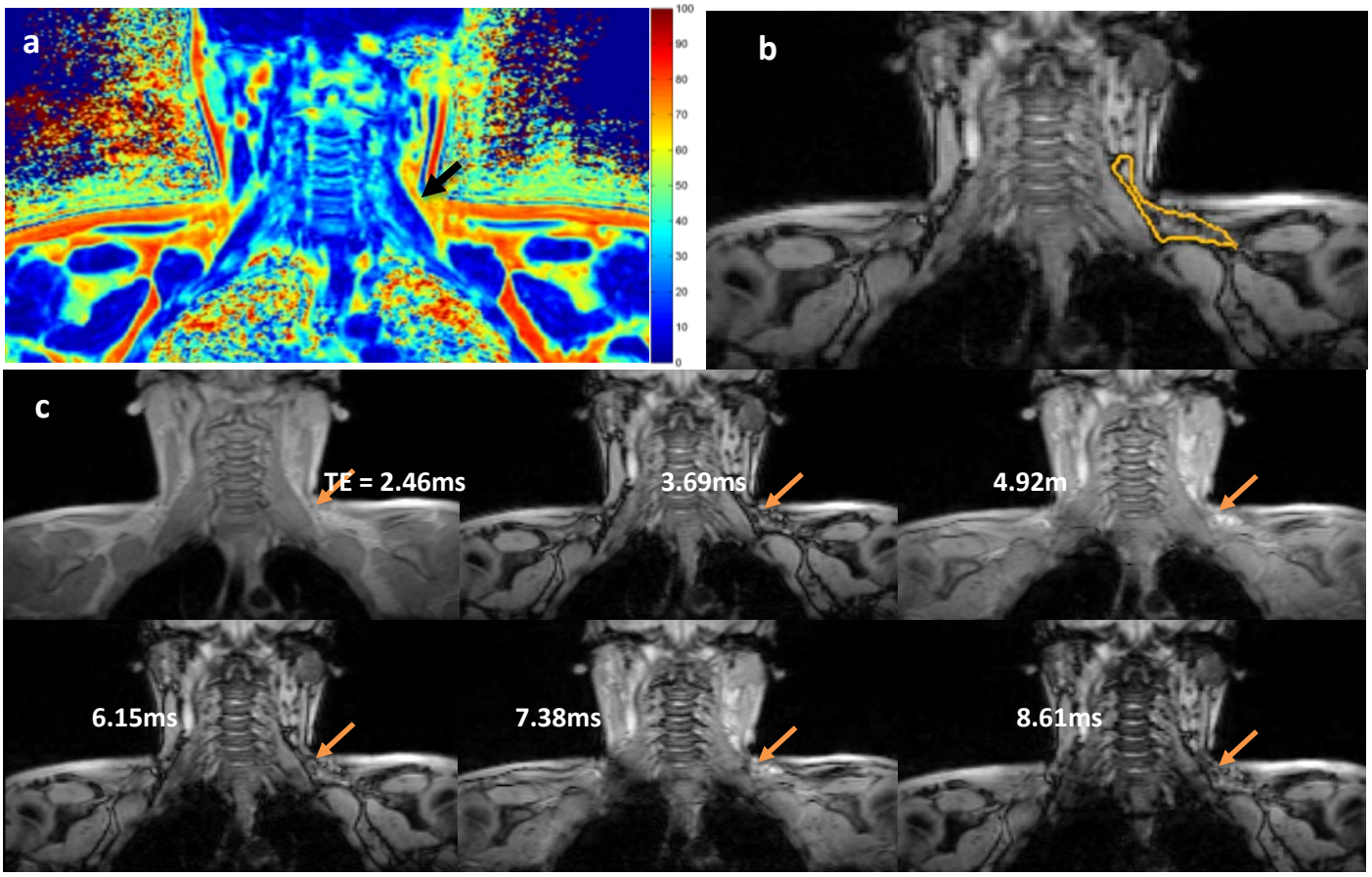
had significantly higher V_{fast} than the pubertal obese group ($V_{fast,G4}$=37.1±3.5%). Interestingly, pubertal lean group ($V_{fast,G3}$= 39.1±5.1%) had lower V_{fast} than the prepubertal obese group G2 (**Fig.3e**).

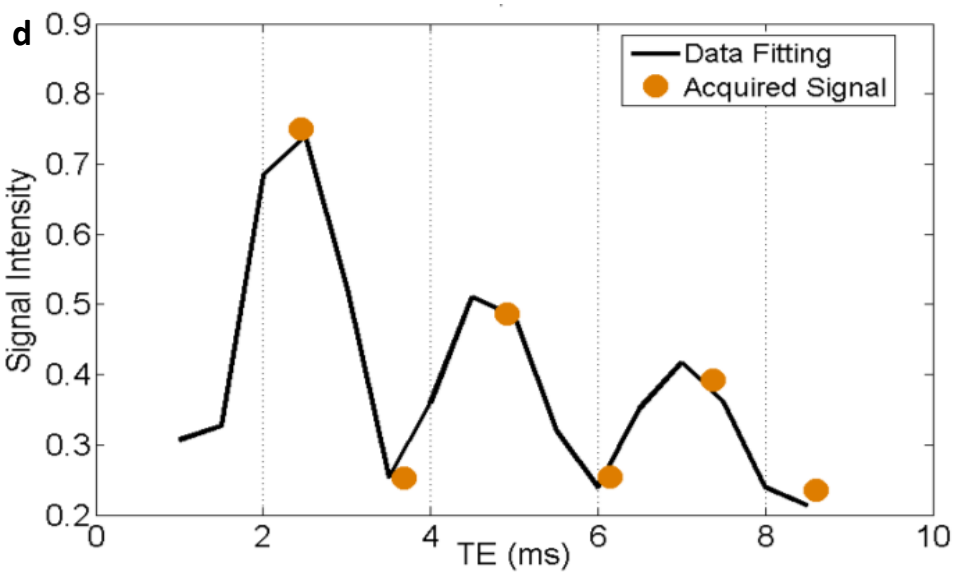
Discussions: In this pilot study we exploited multiple MRI parameters to characterize BAT in pediatric subjects, and identified how BAT tissue properties are affected by obesity and pubertal status. FP and T2* are more sensitive to BAT composition variations associated with obesity and puberty. It demonstrated that BAT in obese and pubertal subjects tends to show more 'WAT-like' properties with increasing FP and T2*. Diffusion parameters of adipose tissues are first used to investigate the adipose tissue water mobility. There was no difference in ADC of BAT between all groups although a significant difference was observed between BAT and WAT (data was not shown in this abstract). The fast diffusion compartment volume which measures the capillary perfusion volume showed that BAT in prepubertal groups have increasing microvasculature compared to pubertal groups. It was not obvious how obesity is related with the BAT microvasculature. The limitation in this study is the smaller sample size of the prepubertal lean group. A larger scale study will be needed to further investigate the effects of obesity, pubertal status and gender on BAT tissue properties**.**

Acknowledgement: This study is supported by The Center of Obesity Management and Prevention (COMP) and The Williams Heart Foundation (WHF).

Reference: [1] van Marken Lichtenbelt WD, et al N Engl J Med. 2009 360(15):1500-8. [2] Shingo B, et al. J Nucl Med 2010 51(2):246-50. [3] Lee P, et al. Int J Obes 2013

Figure 1





(a) Initial fat percentage (FP) map for identification of BAT regions, which is used as a reference for placement of region-of-interest (ROI) on SPGR image (b). Averaged signal intensity within each BAT ROI (c) shows TE-dependent oscillations (d) due to fat and water interference, which are fitted to calculate FP and T2*.

Figure 2

Step 1:

Calculate initial T2* using the first and second IP signals (S_{IP1} and S_{IP2}):

$T2^* = 2.3 \cdot \log (S_{IP1} / S_{IP2})$

⇩

Step 2:

2.1 Set initial guess value of ρ_f=0.6 with prior knowledge

2.2 T2* calculated from Step1 is used as the initial guess value for $T2^*_{eff}$

2.3 Perform Levenberg-Marquardt non-linear fitting based on

$$S(TE) = \left| \rho_{w1} + \rho_{f1} \sum_{n=1}^{Np} C_n \cdot \exp(2\pi i \cdot f_n \cdot TE) \right| \cdot \exp(-TE / T2^*_{eff})$$

⇩

Step 3:

3.1 $\rho_f = \rho_{f1}/(\rho_{f1} + \rho_{w1})$ calculated from Step 2 is used as the initial guess value for ρ_{f2}

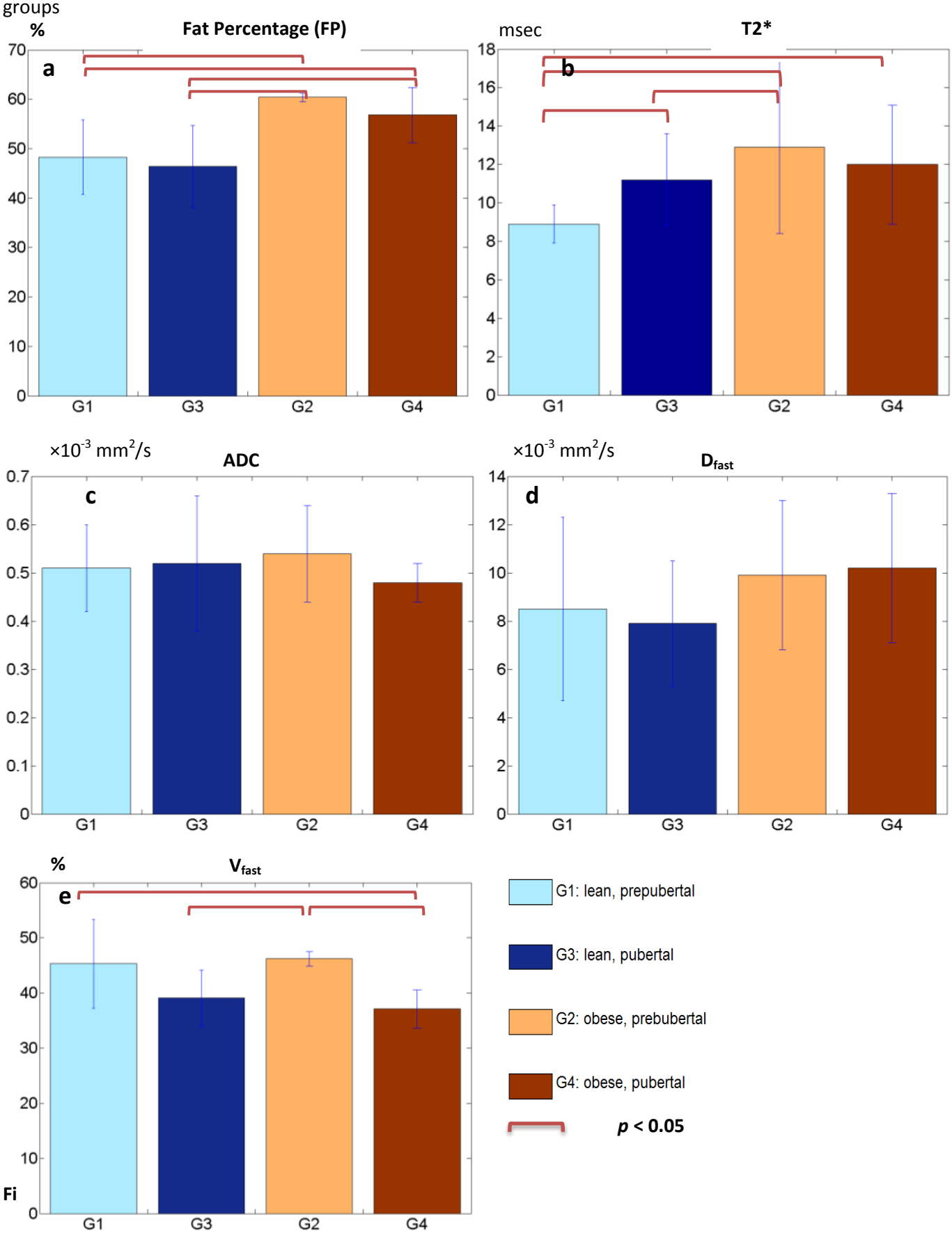
3.2 $T2^*_{eff}$ calculated from Step2 is used as the initial guess value for $T2^*_w$ and $T2^*_f$

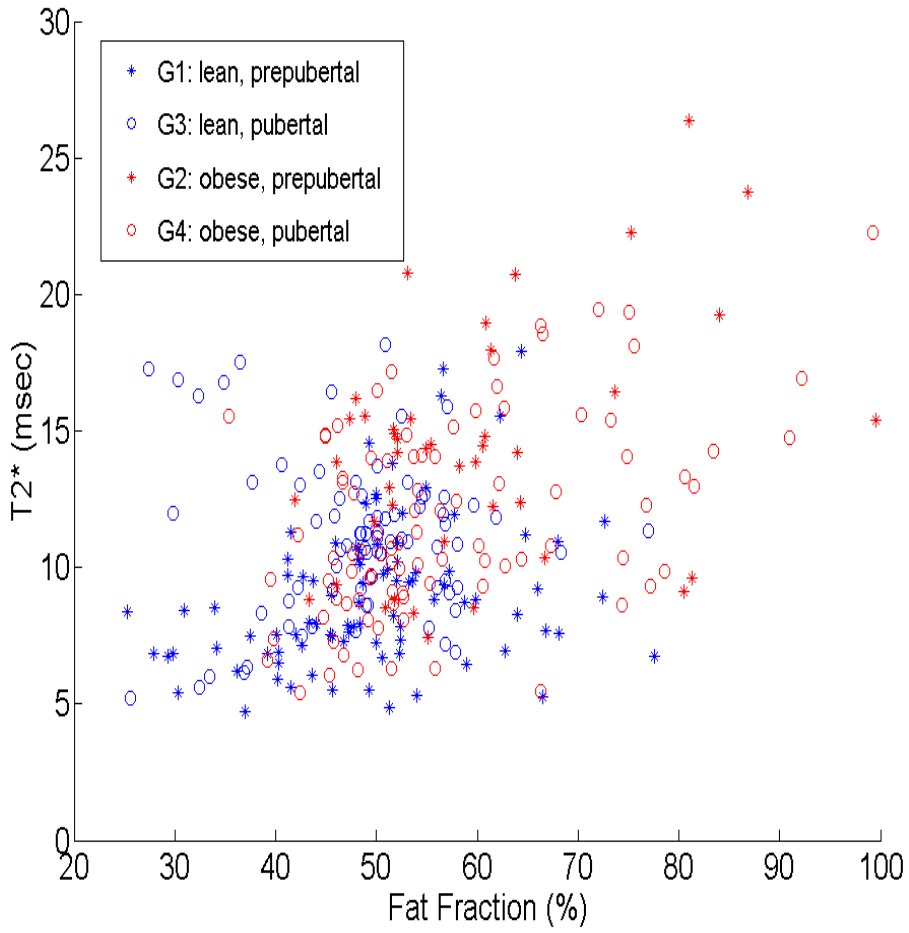
3.3 Perform Levenberg-Marquardt non-linear fitting based on

$$S(TE) = \left| \rho_{w2} \cdot \exp(-TE / T2^*_w) + \rho_{f2} \cdot \exp(-TE / T2^*_f) \cdot \sum_{n=1}^{Np} C_n \cdot \exp(2\pi i \cdot f_n \cdot TE) \right|$$

to calculate ρ_{w2}, ρ_{f2}, $T2^*_w$ and $T2^*_f$

Figure 3: MRI characteristics (**a**: FP, **b**: T2*, **c**: ADC, **d**: D_{fast} and **e**: V_{fast}) comparison between four groups





A scatter plot of the joint distribution of FP and T2* in all four groups.

Poster #14

Imaging of Brown Fat Metabolism Using Hyperpolarised Carbon-13 MRI*

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Introduction: Carbon-13 (^{13}C) enriched substrates can be hyperpolarized via dynamic nuclear polarization (DNP) [1], injected into animals, and imaged at high signal to noise ratios using magnetic resonance imaging. This provides a novel method for studying relatively fast metabolic processes *in vivo*. It has recently been shown that hyperpolarized ^{13}C MRI can be used to measure, in real time, the metabolism of pyruvate in brown fat in rats [2]. In addition, a recent study used pharmaceuticals to increase brown fat metabolism in mice [3], but monitoring of this treatment was not done in vivo. We hypothesize that hyperpolarised ^{13}C MRI may provide an in vivo method for monitoring the effects of pharmaceutical interventions on brown fat metabolism in mice.

Purpose: To use hyperpolarised [1-^{13}C]pyruvate magnetic resonance imaging to detect metabolism in the brown fat of mice before and after metabolic stimulation.

Methods: C57BL/6J mice were fed a normal chow diet (N=1) or a high fat diet (40% fat, N=4) for six weeks, under a protocol approved by the institution's Animal Use Subcommittee. Mice were anesthetised using isoflurane and placed on a custom built surface ^{13}C RF coil inside a custom built switch tuned $^{13}C/^{1}H$ bird cage coil. ^{1}H MRI and ^{13}C spectroscopic imaging were performed in a 3T clinical MRI scanner (Discovery MR750, GE Healthcare, Waukesha, WI, USA). ^{1}H water-fat imaging was used to identify brown fat as described by Hu *et al* [4]. [1-^{13}C]pyruvic acid [Sigma Aldrich] was polarised using a DNP polariser (Hypersense, Oxford Instruments, Abingdon, UK). 0.25mL of the hyperpolarised solution (125mM pyruvate) was injected via the tail vein over 12s. Time resolved 3D ^{13}C images were acquired using a modified IDEAL pulse sequence [5] (7mm isotropic resolution, readout bandwidth= ±6 kHz, flip angle=15° lactate/bicarbonate, 3° pyruvate). Acquisition started 10s after the beginning of the bolus injection and images were acquired every 10s out to 60s. Each mouse received two injections of [1-^{13}C]pyruvate: one before and one 10 min after intraperitoneal injection of isoproterenol (1mg/kg), which stimulated brown fat metabolism.

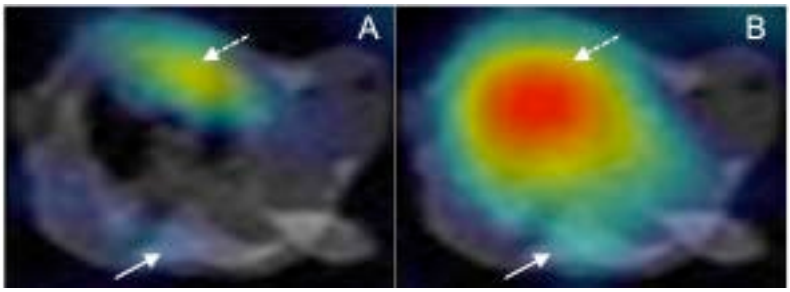

Figure: Overlay of ^{13}C-bicarbonate images 20 seconds post injection, on T_1 weighted axial images pre (A) and post (B) stimulation of metabolism by isoproterenol. (Solid arrows: subscapular fat pad, dashed arrows: heart.) The increased signal post stimulation indicates increased aerobic metabolism.

Results and Discussion: Injection of isoproterenol caused increased blood flow to the subscapular brown fat and was observed by approximately a two fold increase in pyruvate signal as compared to the signal pre-injection of isoproterenol. The lactate signal intensity also doubled after injection of isoproterenol due to increased anaerobic metabolism. The figure shows overlays of the bicarbonate signal on a T_1 weighted axial image, before (A) and after (B) metabolic stimulation. While there is a small amount of bicarbonate present in the heart prior to stimulation, virtually no bicarbonate is seen in the subscapular fat. Post-stimulation, however, bicarbonate is seen in the subscapular brown fat, indicating aerobic metabolism.

Conclusions: Using hyperpolarised ^{13}C MRI, metabolism of [1-^{13}C]pyruvate to lactate and bicarbonate was seen in the subscapular brown fat after stimulation of metabolism with isoproterenol. These results suggest that hyperpolarised [1-^{13}C]pyruvate MRI may be used to monitor the effects of pharmaceutical interventions on brown fat metabolism in mice.

Acknowledgements: We acknowledge Tim Scholl and Patrick Lim for RF coil development and support from GE Healthcare, NSERC, the Ontario Research Fund and the Canada Research Chairs Program.

References: [1] Tam *et al.* Circulation 2012;125:2782. [2] Syn WK *et al*. Liver Int 2009;29(8):1262. [3] Mitschke *et al.* FASEB J 2013;27:1621. [4] Hu *et al.* JMRI 2010;31:1195. [5] Wiens *et al.* Proc ISMRM Workshop on Data Sampling and Image Reconstruction 2013.

*Abstract chosen for a Hot Topic talk

Poster #15

Fat-Water MRI Properties of Brown Adipose Tissue in Adult Humans Using Automated Depot Segmentation Based on Cold-Activated and Thermoneutral PET-CT

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Purpose: To report the quantification of fat-water MRI (FWMRI) properties of brown adipose tissue (BAT) in six adult human subjects using regions of interest determined by automated segmentation methods based on repeated ^{18}F-FDG PET-CT exams performed following both cold-activated and thermoneutral conditions.

Materials and Methods: The study was approved by the local ethics committee to recruit healthy adult subjects to undergo FWMRI and PET-CT scans. Both FWMRI and PET-CT scans were performed twice, each scan on a separate day, once after two hours of exposure to cold conditions (16°C (62°F)) and once after two hours of exposure to thermoneutral conditions (24°C (76°F)). Six subjects (4 males, 2 females, age range: 21 to 41 years) were scanned on a 3.0 Tesla Achieva MRI scanner (Philips Healthcare, Best, The Netherlands), equipped with two-channel parallel transmit capability, a 16-channel Torso-XL surface coil and an X-tend tabletop (X-tend ApS, Hornslet, Denmark). FWMRI was acquired using a multi-stack, multi-slice, mFFE acquisition with 7 stacks of 20 axial slices. Slices were contiguous with a 0 mm gap between slices. Scanner software was modified to enable the acquisition of 8 echoes acquired as two interleaved sets of four echoes with TE1=1.024 ms and effective ΔTE = 0.779 ms. Other acquisition protocol details included: TR = 83 ms, flip angle = 12°, water fat shift = 0.323 pixels, readout sampling bandwidth = 1346.1 Hz/pixel, axial in-plane field of view = 520 mm × 408 mm, acquired voxel size = 2 mm x 2 mm x 7.5 mm, and sensitivity encoding (SENSE) parallel imaging factor = 3. Three-dimensional water/fat separation and R2* estimation based on a multi-scale whole-image optimization algorithm [1] implemented in C++ was performed for each individual slice stack. Fat was modeled using 9 peaks [2]. The first echo of each 4-echo train was discarded to avoid potential contamination of eddy current in the complex water-fat signal model. PET/CT images were acquired from crown to upper-thigh, with 335 slices and an acquired voxel size of 5.47 mm x 5.47 mm x 3.27 mm, and 1.36 mm x 1.36 mm x 3.27 mm respectively.

Following the cold exposure, BAT activation was observed on the PET/CT scans for all six subjects. The four imaging sessions were then coregistered, and CT Hounsfield units (HU) and PET standardized uptake values (SUV) were used to define a BAT region of interest (ROI) without manual input. The following rules were used for automated generation of the BAT ROI: CT HU values: [-200 to -1], cold-activated PET > 2.0 SUV, and the ratio of cold-activated PET SUV to thermoneutral PET SUV > 0.5. The BAT ROI was refined by requiring that more than 15 immediately neighboring pixels (in 3D) must be

part of the initial rule-based ROI. These ROIs were applied to the coregistered FWMRI and PET/CT images. Fat signal fraction (FSF), R2* relaxation rate, SUV and HU values were measured within the BAT ROI for each subject.

Results: Focusing on a range of eight to fifteen slices (depending on the size of activation per subject) in the supraclavicular area, the mean value (95% confidence interval half-width) for the six adults with PET-confirmed cold-activated BAT under thermoneutral conditions were: CT HU -67.8 (6.9), PET SUV 0.8 (0.5), FWMRI FSF 54.6% (6.9%), and FWMRI R2* 127.9 s^{-1} (16.1 s^{-1}). Under cold-activated conditions, the BAT ROI values were: HU -57.7 (3.3), SUV 5.8 (2.4), FSF 51.9% (4.4%), and R2* 125.5 s^{-1} (30.4 s^{-1}). The HU, SUV, FSF and R2* values for white adipose tissue (WAT) ROI in the subcutaneous tissue of the lower back were -89.7 HU (8.1 HU), 0.4 SUV (0.1 SUV), 85.8% (2.5%), and 70.6 s^{-1} (19.3 s^{-1}) respectively under thermoneutral conditions, and -85.3 HU (10.0 HU), 0.6 SUV (0.3 SUV), 87.3% (4.3%), and 70.8 s^{-1} (13.2 s^{-1}) respectively under cold-activated conditions.

Conclusion: We conclude that FWMRI FSF and R2* values can differentiate between WAT and BAT that is confirmed by cold-activated PET-CT. As reported previously for small animals and newborn human subjects [3,4], the FSF is lower in BAT compared to WAT, and the R2* is larger in BAT compared to WAT. Furthermore, the carefully validated FSF and R2* for BAT reported here can form the basis for a BAT classifier based only on MRI that can detect BAT without the detrimental radiation associated with a PET-CT scan.

References: [1] Berglund J, et al. MRM 67(6):1684-93; 2012. [2] Hamilton G, et al. NMR Biomed 24:784-790; 2011. [3] Hu H, *et al.* JMRI (2010) 31(5). [4] Hu H, *et al.* JMRI (2012) 35(4).

Poster #16

Remodeling of White Fat by PPARgamma Deacetylation Acquisition of Brown Fat Characteristics by White Adipose Tissue in Phosphodiesterase 3B Knockout Mice: Genetic Background Matters

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*equal contribution

Transformation of white adipose tissue (WAT), depicted as a “lipid sink” involved in excessive energy storage, into fat-burning brown adipose tissue (BAT), has attracted considerable attention as a promising strategy to treat obesity and related disorders. A cAMP-hydrolyzing enzyme, phosphodiesterase 3B (PDE3B), links insulin- and cAMP-signaling networks in tissues involved in energy metabolism, including WAT. Based on our previous observations that PDE3B ablation lead to clear BAT-like characteristics in epididymal WAT (EWAT) of SvJ129 but not C57BL/6 mice, we utilized PDE3B KO mice generated in these two strains to study mechanisms and impact of genetic background on murine WAT-BAT transformation. We examined adipogenic and thermogenic gene and miRNA expression profiles, distribution of brown and white adipocyte progenitors, as well as the presence of active BAT as measured by radiolabeled fluorodeoxyglucose uptake. We found that ablation of PDE3B in SvJ129 EWAT resulted in elevated expression of genes associated with thermogenesis and mitochondrial function, as well as in higher BAT progenitor content and metabolic activity as compared to WT EWAT. On the other hand, such clear phenotypic changes were not observed in C57 PDE3B KO EWAT until after treatment with the β-adrenergic agonist, CL316243, which markedly increased the expression of genes associated with BAT recruitment in WAT. These data suggest that acquisition of BAT characteristics by PDE3B KO EWAT uses different mechanisms depending on the mouse genetic background.

Poster #17

Fasting-Induced Insulin Resistance Reduces Brown Adipose Tissue Activity[#]

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Background: Human brown adipose tissue (BAT) activity has been associated with non-shivering thermogenesis (NST) and it has been shown that human BAT is a highly insulin sensitive tissue. Retrospective human studies have suggested that type 2 diabetes is associated with reduced BAT activity, measured as BAT glucose uptake. However, it is not known whether BAT glucose uptake is impaired due to insulin resistance, an important physiological hallmark of type 2 diabetes, per se. Therefore, insulin resistance was induced by a 2-day fasting period and its effect on cold-induced BAT glucose uptake and NST was investigated. Previous research has shown that such prolonged fasting periods cause insulin resistance in all young, healthy subjects.

Methods: 16 healthy subjects (8M, 8F, age 21.9±3.1 years, BMI 21.4±1.6 kg/m^2) participated in this randomized cross-over study. BAT activity was measured twice: After a normal-fed period and after a 54-h fasting period (to induce insulin resistance). Cold-induced BAT activity was measured using dynamic ^{18}FDG-PET/CT scanning at maximal NST, as achieved by an individualized cooling protocol. Maximal NST was defined as the increase in energy expenditure above basal metabolic rate, at a temperature just above an individual's shivering temperature. Skin perfusion and skin- and core-temperatures were monitored as well.

Results: In the fasted condition cold-stimulated BAT glucose uptake rate was significantly decreased compared to the control condition (2.7±1.7 vs. 6.0±3.4 µmol/min/100g resp., $p<0.001$). No change in [^{18}F]FDG uptake was observed in other tissues, such as skeletal muscle, liver and brain, upon fasting. NST was also significantly reduced from 12.7±9.1 to 6.7±5.1% ($p<0.01$). Mean skin temperatures and skin perfusion during cold exposure were not different between the normal-fed and the fasted condition. Core temperature showed a significant decrease upon cold exposure in the fasted condition only (-0.26±0.21 °C, $p<0.01$). The difference in core temperature during cold exposure between the fasted and normal-fed condition was significantly correlated to the difference in BAT glucose uptake between these two conditions ($r^2 = 0.350$, $p<0.05$).

Conclusion: Fasting-induced insulin resistance hampers BAT activity, which is accompanied with a reduced non-shivering thermogenesis. If BAT activity is also reduced

in other insulin-resistant conditions, e.g. type 2 diabetes, the reduced glucose uptake in BAT may be involved in the development of hyperglycemia. The relation between BAT activity and core temperature indicates a prominent role for BAT in maintaining a constant core temperature upon cold exposure in humans.

Research related to this abstract was financed by EU FP7 project DIABAT (HEALTH-F2-2011-278373 to W.D. van Marken Lichtenbelt).

[#]Abstract chosen for a travel award

Poster #18

Feasibility of Brown Adipose Tissue Histology Co-registration with 3D Whole Mouse MR Images

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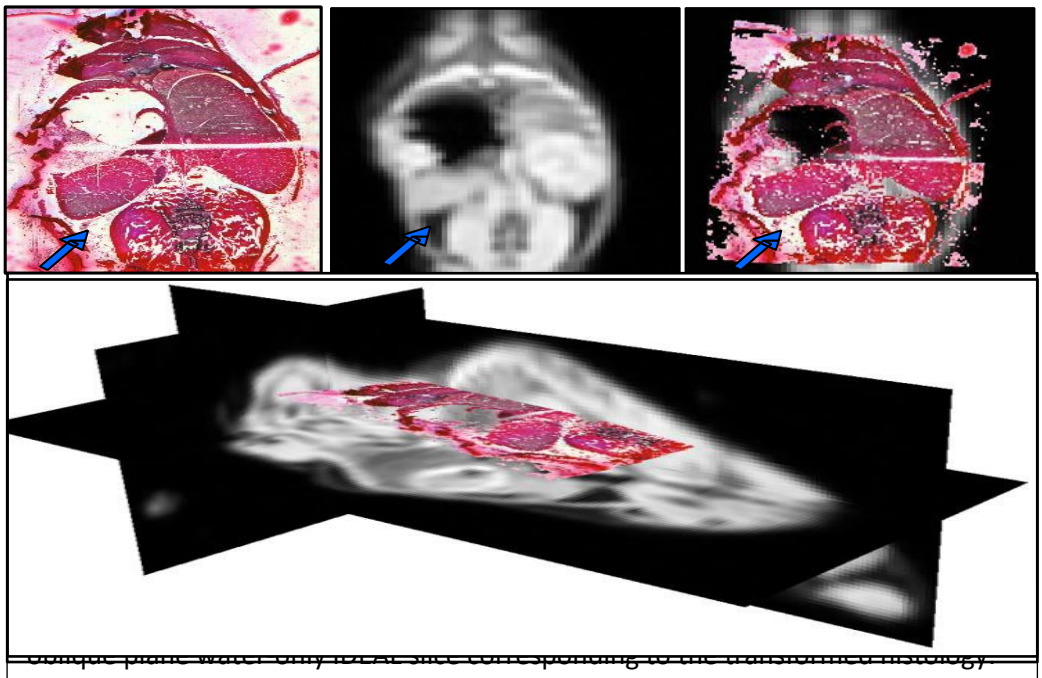
Introduction: Histological preparation limits the ability to perform longitudinal studies on volumetric and metabolic changes to brown adipose tissue (BAT). *In vivo* imaging techniques utilizing MRI have the potential to provide complementary information to histology without the need for animal sacrifice. Validation of such techniques would ideally involve direct correlation with histological data, which may be accomplished by accurate registration. The purpose of this abstract is to demonstrate the feasibility of accurate registration of histology to 3D whole mouse MRI volumes.

Methods: *Animals:* Four 129/SvJ mice were imaged under a protocol approved by the institution's Animal Use Subcommittee. *MRI:* A 45 min 3D quantitative IDEAL MRI acquisition was performed with approximately 0.7 mm isotropic resolution in a 3T MRI scanner. To minimize substantial geometric deformation of the tissue that occurs between MRI scanning and cryo sectioning mice were euthanized immediately preceding scanning allowing rigor mortis to set in, greatly reducing deformation. Optical images: After MR imaging, mice were immediately embedded in an optimal cutting temperature medium and flash frozen in liquid nitrogen. The frozen volume was serially sectioned by a CryoVizTM cryo-imaging system (BioInvision, Cleveland Ohio, USA). High resolution optical images were acquired with an in-plane resolution of 17 µm and slice spacing of 200 µm. Stained sections: Tissue sections were collected using a tape transfer system (Section-lab Co., Yokohama, Japan), at 20 µm thickness, stained with hematoxylin & eosin and digitized (0.5µm/pixel) on a TISSUEScope scanner (Huron Technologies,Waterloo, Canada). Registration: Histological sections were interactively registered to the corresponding planes of the 3D optical image using 2D thin-plate spline (TPS) transformation.The 3D optical images were interactively registered to MRI using a 3D T transformation. Target registration error (TRE) was quantified as the post-registration Euclidean distance (2D TRE for the histology-optical image registration and 3D TRE for the optical-MRI registration) between anatomically homologous landmarks in images using leave-one-out cross-validation.

Figure 1



(C) Image B with transformed histological overlay. (D) 3D view of MRI volume with registered 2D histological slide. Blue arrows in A-C indicate the same region of identifiable white adipose tissue

Results and Discussion: Figure 1 shows co-registration between a single 2D histological slide and 3D water separated MRI. Agreement of registered histology and corresponding oblique plane MRI can be seen in Figure 1B-C. The mean TREs were 0.1 mm for 2D histology to optical images (registered with 50 landmark pairs) and 0.9 mm for 3D MRI to optical volumes (registered with 20 landmark pairs). We have been able to successfully identify white adipose tissue (WAT) in H&E stained histological sections, indicated by the blue arrow in figure 1A and C. This region in the registered histological image aligns with the area of signal void in the water separated MRI in figure 1B, also indicated by a blue arrow. In the future we aim to incorporate immunohistological staining for UCP-1 to our histological slide processing to positively stain for BAT.

Conclusion: Here we successfully demonstrate co-registration of histological sections with 3D MRI; a mean TRE of 0.9 mm was calculated for registration of MRI to optical volumes and 0.1 mm for the registration of histological to optical

Poster #19

UBC9 is a PPARγ Rheostat that Mediates Browning in Human Adipocytes

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We identified the small ubiquitin-related modifier (SUMO) E2-conjugation enzyme Ubc9 as a novel inhibitor of energy storage in human adipocytes using a high-throughput microscopy screen. Subsequent experiments revealed Ubc9 expression to be increased in white adipose tissue from diabetic mice and humans, implying a role for Ubc9 in the regulation of energy balance. Consistent with the idea that increased Ubc9 expression is associated with deregulation of energy balance, overexpression of Ubc9 in human adipocytes blocked induction of mature adipocyte marker genes. Immunoprecipitation and FRET experiments showed thiazolidinediones reduce the interaction between Ubc9 and PPARγ, further suggesting Ubc9 represses PPARγ activity. Moreover, activation of PPARγ using rosiglitazone in human adipocytes decreased Ubc9 expression. Therefore, we hypothesized Ubc9 functions as a rheostat which varies resistance of white adipocytes to anti-diabetic ligands. In loss-of-function experiments performed in human adipocytes, Ubc9 siRNA increased the levels of PPARγ protein and its downstream target genes resulting in enhanced mitochondrial biogenesis and induction of brown adipocyte selective genes. Also, UCP1 mRNA and protein increased upon Ubc9 knockdown, resulting in a significant increase in total and uncoupled respiration. Together, these experiments suggest Ubc9 is a potent repressor of browning in human subcutaneous adipocytes. De-repression of PPARγ through inhibition of Ubc9 represents a novel therapeutic approach in the treatment of type 2 diabetes.

Poster #20

Genome-wide Profiling of Brown Fat-Specific Open Regulatory Regions Identifies NFIA as a Transcriptional Regulator of Brown Fat–Muscle Cell Lineage Specification.

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Brown fat of human and rodents dissipate energy as heat and an important target of treatment for obesity. We performed genome-wide FAIRE-seq on murine brown and white fat tissues to map open chromatin regions that control cell-type specific gene expression. We identified multiple brown fat-specific open chromatin regions near genes, such as Ucp1, Cidea, Pparg1a and Prdm16. Globally, about half of those regions are binding sites for PPARγ, while the rest was not bound by PPARγ. One of the most enriched binding motifs in nucleotide sequence of the brown fat-specific open chromatin regions—besides those for C/EBP and EBFs—was that for a transcription factor NFIA. NFIA is expressed abundantly in both brown and white fat cells but expressed at low levels in muscle cells. Retroviral expression of NFIA in C2C12 muscle cells results in loss of muscle phenotype and induction of brown fat phenotype characterized by formation of lipid droplets and induction of genes such as Ucp1 and Pparg1a that is further responsive to cAMP stimulus. Stimulation of brown fat gene program appears to be lineage-sensitive because overexpression of NFIA in 3T3-L1 cells does not show the browning effect. Co-expression studies of NFIA and PPARγ in C2C12 cells indicated that NFIA not only stimulates brown fat-specific genes but also represses white fat-specific genes independently of its adipogenic effect. Finally, expression levels of brown fat-specific genes are reduced in BAT of NFIA-/- mice. Together, global profiling of brown fat-specific regulatory regions by FAIRE-seq is useful in identifying the role of NFIA in brown fat and muscle cell lineage specification.

Poster #21

mTORC2 Facilitates Brown Adipocyte Differentiation by Suppressing PDGFR Signaling and Promoting SREBP1c Activity[#]

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mTOR complex 2 (mTORC2) phosphorylates the hydrophobic motif (HM) of AKT but its function is poorly understood. By studying time-dependent effects of inhibiting mTORC2 in fibroblasts and brown preadipocytes, we find that pan-AKT signaling acutely requires mTORC2 but that AKT rapidly adapts by uncoupling T-loop (T308) phosphorylation from dependency on HM (S473) phosphorylation. This quickly restores AKT signaling to many of its predicted substrates (e.g. TSC2, GSK3β and PRAS40) and downstream pathways including mTORC1 and S6K activity. However, brown preadipocytes that recover from mTORC2 loss are completely incapable of adipogenesis. This appears to be due at least in part to a failure to decrease PDGFR signaling through an AKT1 - FoxO1 dependent mechanism. Importantly, inhibiting PDGFR signaling restores expression of PPARγ and Ucp1 in mTORC2-deficient brown preadipocytes; however, it only partially restores differentiation because of a secondary defect in the maturation of SREBP1c. Thus, unlike other AKT-regulated processes, mTORC2-dependent AKT hydrophobic motif phosphorylation appears to have a unique and essential function at multiple steps of brown adipocyte differentiation.

To investigate the role of mTORC2 in vivo in adipocyte development, we conditionally deleted *rictor*/mTORC2 with *myf5*-cre. We find that both the brown and white adipocyte lineages derived from Myf5-positive precursors require mTORC2 for normal development in vivo. Specifically, mTORC2-deficient brown and white adipocytes are smaller, have decreased lipid accumulation, and decreased levels of mature SREBP1c. Thus, in vivo mTORC2-deficient preadipocytes appear capable of overcoming the PDGFR-signaling block during differentiation but not the ability to fully activate SREBP1c. Interestingly, mTORC2-deficient brown adipose tissues also have increased expression of *ucp1* mRNA and mitochondrial makers, suggesting mTORC2 might negatively regulate brown fat

activity. Thus, while many AKT-regulated processes can escape their mTORC2 dependency following inhibition of the complex, these results argue that mTORC2 is critical for adipose tissue growth and function.

[#]Abstract chosen for a travel award

Poster #22

Imaging Brown Fat in Humans with [11C] MRB, a Selective Norepinephrine Transporter PET Ligand[#]

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Background: Brown adipose tissue (BAT) plays a critical role in adaptive thermogenesis and there is growing evidence that BAT may play a role in energy homeostasis in adult humans. Greater understanding of the role of BAT in humans has been limited by the current standard detection methodology, [^{18}F]-2-fluoro-deoxy-D-glucose PET imaging (FDG-PET), which requires cold stimulation. We seek to evaluate a novel, mechanistic approach for BAT detection in adult humans under basal, room temperature conditions by capitalizing on the fact that BAT is strongly regulated by the sympathetic nervous system. We have shown previously that BAT can be detected in rodents under both room temperature and cold conditions with [^{11}C] MRB ((*S*,*S*)-[^{11}C]O-methylreboxetine), a highly selective ligand for the norepinephrine transporter (NET).

Methods: Ten healthy, Caucasian subjects (5 male, 5 female; age 25.0±2.2 years; BMI 21.9±2.0 kg/m^2) were recruited. Total body fat and lean body mass were assessed via bioelectrical impedance analysis. Subjects underwent PET/CT imaging using [^{11}C]MRB under room temperature (RT) and mild cold stimulated conditions (RPCM Cool vest, Glacier Tek, Inc., West Melbourne, FL; enthalpy of cold packs is rated to be 15°C) compared to [^{18}F]FDG PET/CT imaging at RT and mild cold conditions. Subjects were fasting and received all 4 scans within 4 weeks.

Results: As expected with FDG, BAT was well-defined in the mild cold condition, but difficult to identify in the RT. 9 out of 10 subjects had labeled BAT with cold FDG and both RT and cold MRB scans (BAT DVR: RT 0.99±0.3 vs cold 1.1±0.26, P=0.31; DVR(BAT/muscle): RT 2.28±0.8 vs cold 2.41±0.56, P=0.26). Furthermore, [^{11}C]MRB distribution (RT and cold) occurred in the same neck/supraclavicular locations as the cold ^{18}F-FDG scans. There were no gender differences in BAT labeling.

Conclusions: By utilizing a mechanistic approach of targeting the strong sympathetic innervation of BAT, [^{11}C] MRB-PET imaging can label BAT under mild cold as well as basal room temperature conditions.

[#]Abstract chosen for a travel award

Poster #23

Activation and Recruitment of Brown Adipose Tissue by TRPV1 Agonists in Humans

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Brown adipose tissue (BAT) is a site for cold- and diet-induced thermogenesis, and thereby a hopeful target for body fat management. The most powerful and physiological stimulus to activate BAT may be cold exposure. Two-hour cold exposure at 19° C, for example, elicits a marked increase in metabolic activity of BAT and a parallel rise in whole-body energy expenditure (EE). Moreover, repeated cold exposure increases BAT activity and cold-induced thermogenesis (CIT), and concomitantly decreases body fatness (J Clin Invest 2013; 123:3404). These effects of cold are mediated by transient receptor potential channels (TRP) and the sympathetic nervous system. Two types of TRP (TRPV1 and TRPA1) are also activated by some food ingredients, such as capsaicin and its nonpungent analogs (capsinoids), daily ingestion of which was reported to increase energy expenditure (EE) and decrease body fatness both in small rodents and humans. In the present study, to test whether TRP agonists can activate and recruit BAT, the effects of acute and chronic ingestion of capsinoids were examined in men and also in mice.

Healthy male volunteers underwent FDG-PET/CT after 2-h cold exposure, and divided into two groups showing high and low BAT activities. Oral ingestion of capsinoids (9 mg) increased EE in 1-2 h in the high BAT activity group (87.6 ± 15.0 kcal/d), but not in the low activity group (9.8 ± 21.9 kcal/d). Placebo ingestion produced no notable change in either group. Thus, capsinoid ingestion increases EE through the activation of BAT in humans. Next, volunteers with low BAT activities were given capsinoids (9 mg) daily for 6 weeks. CIT after capsinoid treatment (200.0 ± 33.9 kcal/d) was higher than that before the treatment (20.6 ± 43.0 kcal/d) and after placebo treatment (81.0 ± 32.5 kcal/d). As CIT is proportional to BAT activity, our results suggest capsinoid-induced recruitment of BAT. The 6-wk capsinoid treatment caused a slight and insignificant reduction of body fat (-2%). When mice were fed on a diet containing capsinoids, UCP1 expression in BAT was increased and body fat content was decreased. However, the fat-reducing effect of capsinoids was not found in UCP1-deficient mice, indicating a critical role of BAT and UCP1 in the anti-obesity effect of capsinoids.

In conclusion, capsinoids are potential for activation and recruitment of BAT, and would be useful as a practical regimen for body weight management in humans.

Poster #24

Brown Adipose Tissue is Present in Human Newborns with Congenital Hypothyroidism Secondary to Athyreosis

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Background: The transition to a colder extra-uterine environment at birth is regulated by the hypothalamus, sympathetic nervous system, and brown adipose tissue (BAT), and involves complex interactions with thyroid hormone (TH). TH receptors, along with type 2 deiodinase (D2) that converts T4 to T3), are present in brown adipocytes, and their deletion/inactivation leads to impaired BAT thermogenesis in animals. Athyreotic human newborns represent a unique population in which to examine BAT in the absence of TH.

Methods: Newborn infants with congenital hypothyroidism were recruited from the Pediatric Endocrinology clinic following identification of an abnormally elevated thyroid stimulating hormone (TSH) level by the California newborn screening program. Prior to thyroxine (T4) replacement, each infant underwent a physical exam, blood analytes [free T4, TSH, thyroglobulin, and a magnetic resonance (MR) imaging scan to assess thyroid dysgenesis and provide BAT measures. Chemical-shift water-fat MR technique was used to obtain quantitative measurements of fat and water content (fat fraction; FF) in the supraclavicular adipose depot. Four newborns with complete thyroid agenesis had baseline and one follow-up assessment at one to three months of age. Data are presented as mean ± SD; data were analyzed by t-test for paired samples and linear regression.

Results: All four athyreotic infants (5.7 ± 0.5 days old) were born to healthy mothers and were products of full-term pregnancies, with initial visit weights of 3.2 ± 0.6 kg, elevated TSH 445-1220 mIU/L, low fT4 0.27-0.8 ng/dL, and no detectable thyroglobulin (<0.1 ng/mL). At the follow-up visit, weight, TSH, and fT4 values were 4.8 ± 0.4 kg, 5.3 ± 8.2 mIU/L, and 3.3 ± 2.2 ng/dL, respectively. Although all MRI studies depicted BAT in the supraclavicular area at both visits, follow-up exams showed a decrease in BAT (as indicated by an increase in FF) in the supraclavicular fossa; 18.4% ± 7.1 vs. 44.5% ± 14.7; $p = 0.007$. When all exams were analyzed together, there was a strong positive correlation between TSH levels and the FF of the supraclavicular area ($r = 0.78$; $p = 0.02$). In contrast, the FF in the subcutaneous white adipose depot did not significantly change (67.6% ± 12.3 vs. 82.7% ± 4.5; $p = 0.058$) and was not related to TSH levels ($r = 0.38$; $p = 0.34$).

Conclusion: Large amounts of BAT are present in human newborns with thyroid agenesis, which decrease with T4 replacement. The results of the current study also suggest an association between TSH and BAT. Further work is needed to determine whether TSH stimulates thermogenesis via TSH receptors present on brown adipocytes.

Poster #25

Peptide Probes for Targeted Brown Adipose Tissue Imaging*

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The presence of brown adipose tissue (BAT) responsible for thermogenic energy dissipation has been revealed in adult humans and has high clinical importance. Due to limitations of current methods for BAT detection, analyzing the abundance and localization of BAT in the body has remained challenging. Here, we use the mouse model to screen a combinatorial library for probes homing to BAT upon systemic administration. We characterize a peptide (with the sequence CPATAERPC) that selectively binds to the vascular endothelium of BAT, but not of intraperitoneal white adipose tissue (WAT). We show that in addition to BAT, this peptide probe also recognizes the vasculature of BAT-like depots of subcutaneous WAT. Our results indicate that the CPATAERPC peptide localizes to BAT even in the absence of sympathetic nervous system stimulation. Finally, we demonstrate that this probe can be used to identify BAT depots in mice by whole body near-infrared (NIR) fluorescence imaging.

*Abstract chosen for a Hot Topic talk

Poster #26

A Multifunctional Protein EWS is Essential for Early Classical Brown Fat Lineage Determination

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Postnatal brown adipose tissue (BAT) or classical brown fat arise from early muscle-progenitors but how brown fat lineage is determined is not completely understood. In the present study, we show that a multifunctional protein EWS (Ewing Sarcoma) is essential for determining classical brown fat lineage during development. BATs from *Ews*-null embryos and newborn mice are developmentally arrested, displaying very little lipid accumulation and absence of UCP1 expression. In vitro, *Ews* null brown preadipocytes fail to differentiate due to loss of *Bmp7* expression, a critical early brown adipogenic factor. We demonstrate that EWS and its interaction protein YBX1 (Y-box binding protein 1) are required for *Bmp7* expression, as depletion of either *Ews* or *Ybx1* leads to loss of *Bmp7* expression and brown adipogenesis. Complementation of *EWS* restores *Bmp7* expression and addition of recombinant BMP7 efficiently rescues brown fat differentiation in *Ews*-deficient cells. In the absence of *Ews*, BATs and brown preadipocytes ectopically express myogenic genes, suggesting a loss of brown fat determination. Collectively, these results demonstrate that EWS is essential for early brown fat lineage determination. Finally, we provide evidence that *Ews* haploinsufficiency in the mouse attenuates cold- or beta3-agonist-induced brown fat formation.

Poster #27

Brown Fat Dynamics: Elucidation of Molecular Drivers Based on the Innate Expertise of a Hibernator*

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Hibernating ground squirrels are effectively heterotherms, repeatedly cycling between cold torpor with body temperature (Tb) near freezing, and warm euthermia with Tb near 37°C. Maintenance of Tb during torpor and its rapid increase during arousal depend on tight control of non-shivering thermogenesis in brown adipose tissue (BAT). In contrast, summer ground squirrels are active homeotherms, allowing their ample winter BAT reserves to virtually disappear while using this period to reproduce, grow and fatten. Understanding the hibernator's control of these extreme and seasonally distinct recruitment and activation strategies offers untapped potential to identify novel, therapeutically relevant regulatory pathways in BAT. To this end, we collected BAT from ground squirrels across the year, capturing a precisely-timed series of samples that represent the hibernator's distinct seasonal and state-dependent phenotypic transitions. BAT morphology, proteins and transcripts were evaluated for quantitative differences among phenotypic groups. Using samples from three summer and five winter states, as well as mixed physiology fall and spring individuals to test for ambient temperature and seasonal effects, we found that BAT form and function are strongly time-of-year-dependent in these animals, as evidenced by morphology and proteome dynamics. Surprisingly, ambient temperature did not affect the BAT proteome during the transition between summer and winter; rather the pronounced summer-winter shift preceded environmental changes and phenotypic progression. This intrinsic rhythm distinguished among spring and summer homeotherms and among early vs. late winter hibernators. In contrast, the proteome was relatively constant in winter, apparently optimized to exploit lipid fuels by enhanced fatty acid binding, β-oxidation and mitochondrial protein translocation. This constancy was unexpected given the extreme physiological changes encountered as animals cycle between torpor and arousal. Consistent with the protein data, most transcript changes were seasonal; mRNAs involved in lipid transport and metabolism dominated winter heterothermy, while those involved in apoptosis and RNA-processing dominated the period of spring homeothermy. Strikingly, many of the significant transcript changes during winter hibernation exhibited an unexpected pattern – apparently increasing despite low Tb when transcription is suppressed during torpor and early arousal. This finding is exemplified by RPPH1, which appeared to increase 80-fold during torpor. Upon further investigation, we discovered that this normally non-polyadenylated RNA gained a polyA tail during torpor, resulting in its enrichment during mRNA library preparation. We hypothesize that like RPPH1, other torpor-increased transcripts are enriched by additional and/or alternative polyadenylation. Because many of these transcripts are involved in pathways related to BAT metabolic activity, we further postulate that polyadenylation protects essential transcripts from degradation during torpor and sequesters a selected subset for immediate utilization upon arousal.

This work was supported by NIH 1R21DK095180. We also acknowledge the Institutional Proteomics Mass Spectrometry Facility, which is supported in part by NIH Grants to the Colorado Clinical and Translational Science Institute (UL1-RR025780) and the University of Colorado Cancer Center (P30-CA046934).

*Abstract chosen for a Hot Topic talk

Poster #28

Interscapular Brown Adipose Tissue and Adipose Distribution in Magnetic Resonance Images of Mice

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Introduction: Brown Adipose Tissue (BAT) is recognizable in Magnetic Resonance Images (MRI) and the volume and fat fraction of BAT deposits change with total adipose tissue (TAT) volume [1,2]. In addition, Intra-Abdominal Adipose Tissue (IAAT) is predictive of disease rather than TAT or Subcutaneous Adipose Tissue (SAT) [3,4]. We have developed an automated adipose quantification method for human MRI [5] and propose this technique for analysis of interscapular adipose tissue versus TAT, SAT, and IAAT in mouse MRI.

Methods: As approved by the institutional Animal Use Subcommittee, in vivo data were obtained from 7 mice (3 obese, 4 lean). IDEAL (Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation) images were acquired with near-isotropic 500 - 700 µm resolution. TAT, SAT and IAAT were automatically segmented using the method of Addeman et al [5] after minor modification for mice, while the interscapular brown fat pad was manually segmented.

Results: Example segmentations of an obese and a lean mouse are shown in the figure below. From left to right are sagittal fat fraction images, 3D rendered volume of SAT, and 3D rendered volume of IAAT. Automated segmentations of equivalent quality were achieved in 3 obese mice and 4 lean mice with mean TAT volume = 8 ± 1 mL and 0.5 ± 0.4 mL respectively. IAAT volumes were 56 ± 6% for the obese mice and 26 ± 12% for the lean mice. The obese mice had an interscapular fat volume of 0.67 ± 0.02 mL with a measured fat fraction of 83 ± 1%. The lean mice had an interscapular volume of 0.09 ± 0.03 mL with a fat fraction of 45 ± 9%.

Discussion: The modifications from the human method were minor adjustments to thresholds and creating the tissue mask. These were changed due to the large difference in image sizes between mice and humans, causing issues resolving the separation between IAAT and SAT. This demonstrates the algorithm's flexibility and applicability from mouse research to human research. Few minor errors occurred in automated segmentation of tissues in mouse images due to lower resolution in the axial plane. Applications of this method include analysis of correlations between interscapular adipose tissue fat fraction and volume versus distribution of total and abdominal adipose tissue in mice or humans.

Conclusion: This method is feasible for use in studies comparing interscapular adipose tissue and adipose distribution in both small animal models and human research. Monitoring changes within the fat fraction and volume of the interscapular adipose could reveal important information on the effect of brown fat on adipose distribution and weight loss.

[1] J Nucl Med 2013; 54(9):1-4, [2] JMRI 2010; 31:1195-1202, [3] Diabetes Care 2009;

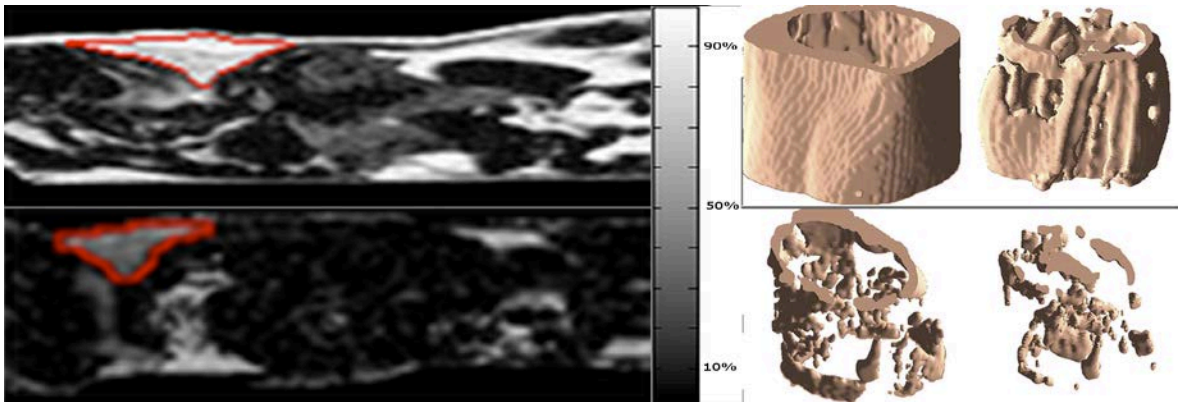

Figure 1. Top: Obese Mouse, Bottom: Lean Mouse.
From Left to Right: Sagittal fat fraction image with fat pad segmentation highlighted in red, 3D render of segmented subcutaneous adipose tissue, 3D render of segmented intra-abdominal adipose tissue. Note that the small volumes of subcutaneous adipose tissue in the lean mouse do not connect around the

32(5):932-937, [4] Obes Rev 2010;11(1):11-18, [5] ISMRM Abstract 4249

Poster #29

Chronic BMP7 Administration Reduces Body Weight, Improves Insulin Sensitivity and Induces a Thermogenic Gene Program in the Inguinal WAT of C57/BL/6J Mice

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Enhancing the development and/or activity of brown adipose tissue (BAT) or the "browning" of white adipose tissue (WAT) is of potential therapeutic benefit for obese individuals. Compelling evidence suggests that Bone Morphogenic Protein 7 (BMP7), a member of the Transforming Growth Factor β (TGFβ) family of secreted proteins, is required for BAT development and initiates the commitment of progenitor cells to a brown adipocyte fate. In this study, we aimed to address the potential role of chronic BMP7 administration on metabolic parameters and thermogenesis ("browning") in WAT of obese C57/Bl/6J mice. 28 day BMP7 treatment resulted in a dose-dependent reduction of body weight in diet-induced obese mice. A small, but significant, change in food intake was observed during the first week of administration, which resolved by the end of the study. A dose-dependent improvement in insulin sensitivity was observed following glucose tolerance testing at Day 24. At the study end, total weight of the inguinal WAT depot decreased by approximately 50% in BMP7-treated animals. Thermogenic (Ucp1), but not adipogenic gene expression (aP2), was increased in the inguinal WAT of treated animals. No changes in classical BAT gene expression or tissue weight were noted. Formation of bony nodules at the site of BMP7 injection is consistent with the retention of BMP7 at a high local concentration versus overall systemic distribution. This study demonstrates the role of BMP7 in the improvement of insulin sensitivity and body weight in obese mice and in the orchestration of a thermogenic gene expression program in subcutaneous WAT. However, challenges must be overcome to prevent the formation of ectopic bone at the site of injection and to improve systemic BMP7 exposure for therapeutic benefit.

Poster #30

Molecular and Functional Characterization of Adult Human BAT Derived from Supraclavicular Tissue

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The imbalance of excess caloric intake and diminished energy expenditure is a major cause of obesity and diabetes in humans. Recent evidences demonstrating that adult humans have functional brown adipose tissue (BAT) depots has introduced the possibility that expansion and activation of BAT could be a new therapeutic approach to combat diabetes, obesity and associated metabolic pathologies. BAT is localized in distinct locations, including the cervical, supraclavicular, and paravertebral areas. There is a growing interest in understanding BAT differentiation and function in humans. Towards this aim, we established immortalized preadipocyte cultures from the supraclavicular region of an adult donor and characterized these cells for their differentiation potential, gene expression and oxygen consumption. We have compared the responses of this cell line to human subcutaneous as well as mouse interscapular brown fat cells. Our human brown adipocytes exhibit expression of a thermogenic transcript profile and oxygen consumption that is similar to mouse classical brown fat. In addition, we show that stimulation with 8-Br-cAMP further enhances thermogenic gene expression and, importantly, elevates uncoupled respiration. In summary, our preliminary data suggests that human-derived supraclavicular brown adipocytes have molecular and functional signatures similar to classical brown fat. While additional characterization is warranted, these cells have the potential to be useful to both explore the physiology of BAT and provide insights on therapeutic opportunities to expand and activate BAT in humans to combat metabolic disorders.

Poster #31

Does Sarcolipin and Muscle Based Thermogenesis Compensate in BAT Deficient Mammals?*

Muthu Periasamy, The Ohio State University, Columbus, OH

Endotherms (Birds and Mammals) must maintain a constant body temperature not only for survival but for optimum physiological functions. Brown adipose tissue (BAT) found in most mammals (absent in birds and pigs) is a major contributor to adaptive thermogenesis. However, it is becoming increasingly clear that other thermogenic mechanisms also exist. Our laboratory's identification of Sarcolipin (SLN) and skeletal muscle as significant players in thermogenesis has challenged the dogma that BAT is the only contributor to non-shivering thermogenesis in mammals. While it's undeniable that BAT is an abundant and powerful heat generator in rodents; BAT is completely absent in birds and its content is minimal in *adult large* mammals, including rabbits, ruminants, and importantly, in adult humans. Studies from our laboratory and others have shown that Sarcolipin (SLN), a regulator of SR Ca^{2+} ATPase (SERCA), can promote uncoupling of SERCA and increase ATP hydrolysis. In the absence of SLN, SERCA utilizes 1 mol of ATP per 2 mol Ca^{2+} transported from the cytoplasm to SR while, in the presence of SLN, SERCA increases ATP hydrolysis and heat production; hence, SLN was proposed to play a role in muscle thermogenesis by promoting futile Ca^{2+} cycling *via* SERCA pump. To better define the physiological significance of SLN in muscle, we took a genetic approach and showed that loss of SLN predisposes mice to develop hypothermia during acute cold exposure but reintroduction of SLN in the $Sln^{-/-}$ background fully restored muscle-based thermogenesis (Nature Med 18, 1575-1579, 2012.). Most importantly, $Sln^{-/-}$ mice gained significantly greater weight compared to WT littermates when fed with a high fat diet (HFD). Interestingly, WT mice fed on HFD showed significant up regulation of SLN (3-4 folds) in certain muscles. Furthermore, mice overexpressing SLN are resistant to diet induced obesity suggesting that SLN/SERCA-mediated thermogenesis is recruited to increase energy expenditure. Generation of DKO mice for SLN and UCP1 further confirmed that SLN is an important player in adaptive thermogenesis. We propose that skeletal muscle is a major site of diet-induced thermogenesis and recruitment of futile Ca^{2+} cycling by SERCA (*via* SLN uncoupling of SERCA) can promote increased ATP hydrolysis and energy expenditure. These studies also suggest that approaches to increase energy expenditure in skeletal muscle could be an important strategy to control weight gain and obesity.

*Abstract chosen for a Hot Topic talk

Poster #32

Thyroid Hormone Receptor Agonists Elicit a Functional Conversion of White to Brown Fat

Jean Z Lin[1,2], Daniel Davila[1,3], Alexandro J. Martagón[1,3], Stephanie L. Cimini[1], Amadeo B. Biter[1], Nan Zhang[1], John D. Baxter[1], Paul Webb[1], Jan-Åke Gustafsson[1,2], Kevin J. Phillips[1]

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Functional conversion of white adipose tissue (WAT) into tissue reminiscent of brown adipose tissue (BAT), often referred to as “beiging”, may be an important strategy to combat obesity and control type 2 diabetes mellitus. We demonstrate that thyroid hormone (T_3) receptor (TR) activation by T_3 or the synthetic agonist GC-1 induces a program of adaptive thermogenesis and uncoupled respiration in subcutaneous WAT that is correspondent with the amelioration of obesity and insulin resistance. Surgical denervation of BAT has no effect on systemic thermogenesis, and all thermogenesis is lost in lean mice that possess little subcutaneous WAT, suggesting that induction of thermogenesis is mediated exclusively by WAT. These data demonstrate that TR activation can elicit facultative thermogenesis in WAT, a previously unrecognized component of TR-mediated thermogenesis, and establish the profound pharmacological potential of WAT beiging.

Poster #33

Remodeling of White Fat by PPARgamma Deacetylation

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Brown adipose tissue (BAT) can disperse stored energy as heat. Promoting BAT-like features in white adipose (WAT) is an attractive, if elusive therapeutic approach to staunch the current obesity epidemic. We have reported that gain-of-function of the NAD-dependent deacetylase SirT1 or loss-of-function of its endogenous inhibitor Deleted in breast cancer-1 (Dbc1) promote "browning" of WAT by deacetylating peroxisome proliferator-activated receptor (Ppar)-γ on Lys268 and Lys293. SirT1-dependent deacetylation of Lys268 and Lys293 is required to recruit the BAT program coactivator Prdm16 to Pparγ, leading to selective induction of BAT genes and repression of visceral WAT genes associated with insulin resistance. In *in vitro* system, an acetylation-defective Pparγ mutant (2KR) induces a brown phenotype in white adipocytes, while an acetylated mimetic (KQ) fails to induce "brown" genes, but retains the ability to activate "white" genes.

We are making the Pparγ acetylation-defective *2KR knock-in* mice to investigate the physiological role of Pparγ acetylation *in vivo*. We propose that its physiological functions are repressing "whitening" as well as inducing "browning" in white adipose tissue. Deacetylation of Pparγ will lead to improved adiposity health without the deleterious side effects as Thiazolidinediones (TZDs) do. In summary, posttranslational modifications (PTMs) of Pparγ will determine its transcriptional selectivity and thus hold the promise of next generation drugs for treating Type 2 Diabetes.

Poster #34

Role of Tyk2 in Regulating Energy Expenditure and Preventing Obesity[#]

Vidisha Raje[1], Ph.D., Marta Derecka[1], Ph.D., Andrew C Larner[1], M.D, Ph.D.

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Obesity develops when energy intake exceeds energy expenditure. Defects in the function of brown fat and skeletal muscle, two of the major tissues that contribute towards energy expenditure, lead to the development of obesity and metabolic syndrome. Mice that lack the tyrosine kinase Tyk2, which is a member of the JAK-STAT signaling family, become obese and develop the metabolic syndrome. Tyk2-/- mice display defects in brown fat development and function. Interestingly, these mice also exhibit abnormalities in the function of skeletal muscle, which is also derived from the Myf5+ lineage of mesenchymal stem cells, as brown fat. The mitochondria in both brown fat and skeletal muscle in Tyk2-/- mice exhibit structural and functional defects, which could contribute to altered energy expenditure. Tyk2-/- mice are not able to maintain their body temperature following acute cold exposure. Tyk2-/- mice also fail to induce UCP1 expression in the inguinal fat following injection with a β3 agonist. Interestingly, when we restored the expression of Tyk2 or kinase-inactive Tyk2 in brown fat and skeletal muscle of Tyk2-/- mice, we were able to revert the obese and the thermogenic phenotype. Our studies also suggest that the kinase activity of Tyk2 is dispensable for its role in brown fat and skeletal muscle function. Rather, Tyk2 interacts with the transcription factors PRDM16 and PGC1α and also binds to the UCP1 promoter, suggesting a nuclear function of Tyk2.

Our studies clearly define a novel role of Tyk2 in regulating thermogenesis and also highlight the role of Tyk2, not as a kinase, but as a component of the transcription complex that regulates the expression of genes involved in brown fat and skeletal muscle differentiation and function.

[#]Abstract chosen for a travel award

Poster #35

Curcumin Analogues as in vivo Fluorescence Imaging Probes for Brown Adipose Tissue and Monitoring Browning

Xueli Zhang,M.S.[1,2], Hongbin Zhang, Ph.D.[3], Yanli Tian, M.S.[1,4], Amol Kavishwar, Ph.D.[1], Matthew Lynes, Ph.D.[3], Yu-Hua Tseng, Ph.D.[3], Anna Moore, Ph.D.[1*], and Chongzhao Ran, Ph.D.[1]

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Brown adipose tissue (BAT), widely known as 'good fat', has recently emerged as an important target for diabetes, obesity and other diseases. Imaging probe that could consistently monitor BAT mass and browning of white adipose tissue (WAT) is highly desirable. However, specific imaging ligands for BAT are few. Therefore, to solve the problem we proposed an unconventional whole-organism fluorescence imaging screening method (a top-down approach) using nude mice for an initial screening. Through the top-down screening of 38 fluorescent dyes, we found that BAT could be successfully imaged in mice with curcumin analogues. Through the lead optimization, we found that CRANAD-29 represents an excellent near-infrared fluorescent imaging probe for BAT with significantly higher selectivity for BAT over WAT. We validated the utility of CRANAD-29 for imaging BAT mass change in a streptozotocin (STZ)-induced diabetic mouse model, and for monitoring BAT activation under cold exposure. In addition, CRANAD-29 could be used for monitoring browning of subcutaneous WAT (sWAT) induced by CL-316,243. We believe that the synthesized imaging probes could serve as useful tools for investigating BAT and browning in preclinical research, and have a tremendous potential for translation into future clinical studies.

Poster #36

In Vivo Optical Imaging of Interscapular Brown Adipose Tissue with 18F-FDG via Cerenkov Luminescence Imaging

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Objective: Recent studies validated the presence of brown adipose tissue (BAT) in human adults, making it an important re-emerged target for various pathologies. In the course of this validation, PET (positron emission tomography) imaging with 18F-FDG played a vital role due to the significant uptake of 18F-FDG by BAT under certain conditions. In this report, we demonstrated that Cerenkov luminescence (CLI) from 18F-FDG could be utilized for in vivo optical imaging of BAT in mice. The shallow location of interscapular BAT also contributes to the feasibility of CLI imaging.

Methods: Mice (nu/nu, n= 4) we injected with 18F-FDG (250 µCi) and imaged 60 minutes later with open filter and 2 min acquisition. In vivo activation of BAT was performed by i.p. injection of norepinephrine (NE) under isoflurane anesthesia.

Results: Our data revealed excellent correlation between in vivo Cerenkov luminescence imaging (CLI), ex vivo CLI and ex vivo radioactive dosimetry counting, suggesting the reliability of this method. 3D reconstruction images obtained by multispectral Cerenkov luminescence tomography showed excellent correlation between the source of the light signal and the location of interscapular BAT. In addition, we showed that this approach could be used to monitor the activation of BAT in mice. Specifically, we demonstrated that the CLI signal in NE-treated group was 2.47-fold higher that in the control group 60 minutes after injection of 150 µCi of 18F-FDG. .

Conclusion: Compared to PET imaging with 18F-FDG, this CLI method is much cheaper and faster. We believe that this method could be used as an important tool for researchers investigating BAT.

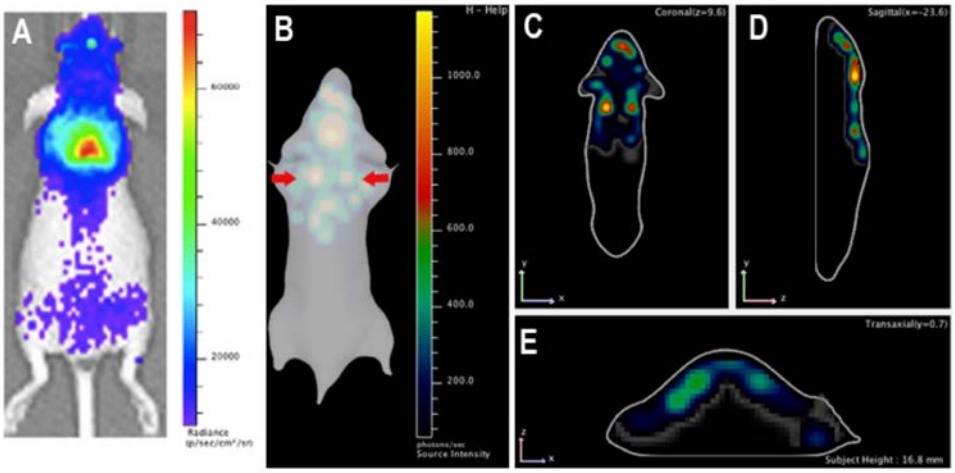


Fig. BAT imaging by Cerenkov Luminescence Imaging (CLI) with 18F-FDG in nude mice. (A) Open filter imaging with 18F-FDG; and (B-E) 3D reconstructed images, coronal view (C), sagittal view (D) and transverse view (E).

Poster #37

BAT Quantification Reliability in Human Neonates Using Water-Fat Separated MRI

Authors: Jerod M Rasmussen MS[1], Sonja Entringer PhD[1,2], Joshua Burns BS[1], Theo GM van Erp PhD[1], James M Swanson PhD[1], Pathik D Wadhwa MD,PhD[1], Claudia Buss PhD[1,2], Steven G Potkin MD[1]. 1.University of California - Irvine. Irvine, Ca. USA. 2. Charité, Berlin, Germany.

Introduction: There is a major resurgence of interest in brown adipose tissue (BAT) biology, particularly regarding its determinants and consequences in newborns and infants. Reliable methods for non-invasive BAT measurement in human infants have yet to be demonstrated. The current study applies existing MRI BAT quantification methodology[1] in a cohort of *in vivo* infants to establish the reliability of these measures.

Methods: Neonatal scans (n=22) were acquired during natural sleep to quantify BAT and WAT deposits for volume and fat fraction. All imaging occurred on a Siemens 3T Tim Trio using a 12 channel phased array receive head and neck coil. A two-point Dixon method[2] was used (TR=7.47ms, TE=2.45ms, FA=10, 1mm isotropic resolution). Fat and water separated images were calculated from the in/opposed phase images collected at their respective TE. Fat fraction (FF) was calculated voxelwise as the ratio of fat to the combined signal contribution from fat and water. Two raters (inter-rater) independently quantified BAT deposits using semi-automated segmentation[3] methods, with the first rater performing two ratings (intra-rater) separated by two weeks in time. Acquisition repeatability was assessed based on multiple scans from the same neonate. Intra- and inter-rater measures of reliability in regional BAT quantification were determined.

Results: Human neonate deposits indicative of bilateral BAT in spinal, supraclavicular and axillary regions were observed. Mean WAT fat fraction was significantly differentiated from mean BAT fat fraction in all subjects ($<\Delta_{WAT,FF-BAT,FF}>=38\%$, $P<10^{-4}$). Repeated scans demonstrated a high voxelwise correlation for fat fraction ($r_{all}=0.99$, Figure 2). BAT volume and fat fraction measurements showed high intra-rater ($ICC_{BAT,VOL}=0.93$, $ICC_{BAT,FF}=0.93$) and inter-rater reliability ($ICC_{BAT,VOL}=0.86$, $ICC_{BAT,FF}=0.93$) (Figure 3).

Discussion: This study demonstrates the reliability of using magnetic resonance chemical shift imaging in human neonates for quantification throughout the torso of BAT volume and fat fraction.

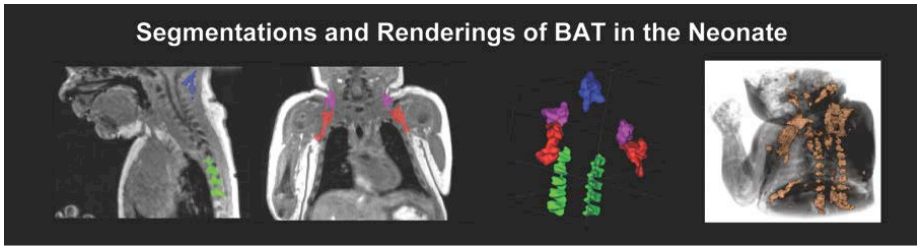
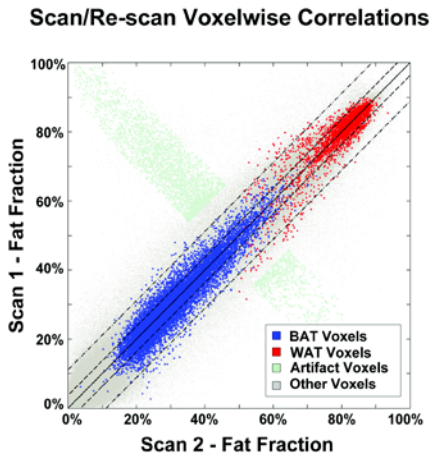


Figure 1. Example bilateral BAT depots in the human neonate, imaged in vivo. Axillary (red), supraclavicular (magenta), and spine (green) are highlighted.

References:

1.Hu et. al. (2013) Comparison of brown and white adipose

tissues in infants and children with chemical-shift-encoded water-fat MRI. *J Magn Reson Imaging*. 2. Lidell et. al. (2103) Evidence for two types of brown adipose tissue in humans. *Nature Medicine*. 3. Yushkevich et. al. (2006) User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage.*
Acknowledgements: This project was supported by NIH grants RO1 MH-091351 to CB,



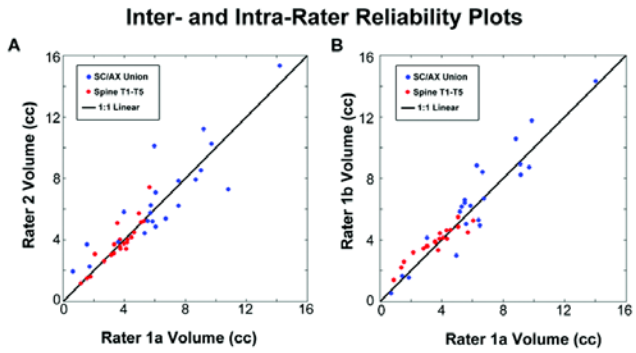


Figure 3 (left). Acquisition repeatability plot demonstrates a repeatable measurement of in vivo human neonatal FF.

Figure 2 (above). Inter- (left panel) and intra-rater (right panel) reliability plots show a high degree of rating reliability.

RO1 HD-065825 to SE, R21 DK098765 to SE and CB, and RO1 HD-060628 to PDW.

Poster #38

Changes in BAT Composition from Birth Through 12 Months of Age in Human Infants: Preliminary Observations[#]

Jerod M Rasmussen-MS[1], Sonja Entringer PhD[1,2], Theo GM van Erp PhD[1], Steven G Potkin MD[1], Pathik D Wadhwa MD[1], Claudia Buss PhD[1,2].

[1]University of California – Irvine, Irvine, California, USA
[2] Charité, Berlin, Germany

Introduction: There is a major resurgence of interest in brown adipose tissue (BAT) biology, including the determinants of its changes from newborn to later stages in life. It is, therefore, of interest to characterize the magnitude of inter-individual variability in intra-individual changes in BAT during development. At least one previous cross-sectional study has documented an age-related increase in BAT content in early life.[1] However, the question of whether the magnitude of this change is predicted by the initial (newborn) composition of BAT has not yet been addressed. From an on-going study we present here preliminary observations of changes in fat fraction in human infants over the first year of life.

Methods: Longitudinal MRI scans were acquired during natural sleep of 7 babies during neonatal (24.6±9.6 days) and infant (381.7±5.2 days) stages of development to quantify supraclavicular/axillary BAT deposit fat fraction. Imagng was done on a Siemens 3T Tim Trio with a 12 channel phased array receive head and neck coil using a two-point Dixon method[2] (TR=7.47ms, TE=2.45ms, FA=10, 1mm isotropic resolution). Fat and water separated images were calculated from the in/opposed phase images collected at their respective TE. Fat fraction (FF) was calculated voxelwise as the ratio of fat to the combined signal contribution from fat and water. Segmentations were done using semi-automated methods[3] and fat fraction estimates were extracted from resulting regions of interest.

Results: Deposits indicative of bilateral BAT in supraclavicular/axillary regions were observed at birth and at 1 year age (Figure 1). Subcutaneous WAT was significantly differentiated from supraclavicular/axillary BAT in all participants ($\Delta_{NB,FF}$=42.1%, $\Delta_{1YO,FF}$=32.6%; $p<10^{-3}$). There was a significant increase in relative BAT FF (mean $\Delta FF_{WAT,Long}$ = 16.0% per year) and WAT FF (mean $\Delta FF_{BAT,Long}$ = 6.1% per year) for all participants (Figure 2). BAT FF at birth predicted 45% of the variation in BAT FF at one year of age ($p_{one\text{-}tailed}$=0.05).

Discussion: Longitudinal measurements of BAT FF indicate substantial variability and significant increases in adipocyte density during the first year of life. It also appears that BAT composition at birth may predict change in BAT over the first year of life. The intrauterine and postnatal determinants and metabolic consequences of such intra-individual variability are the focus of ongoing studies.

References: 1.Hu et. al. (2013) Comparison of brown and white adipose tissues in infants and children with chemical-shift-encoded water-fat MRI. *J Magn Reson Imaging*. 2. Lidell et. al. (2103) Evidence for two types of brown adipose tissue in humans. *Nature Medicine*. 3. Yushkevich et. al. (2006) User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage.*

Acknowledgements: This project was supported by NIH grants R21 DK098765 to SE and CB , RO1 MH-091351 to CB, RO1 HD-065825 to SE, and RO1 HD-060628 to PDW.

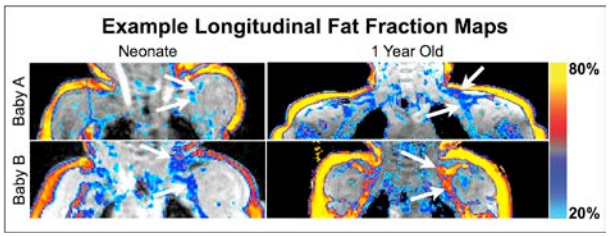


Figure 3. Example longitudinal fat fraction maps. Lower fat fraction represents a mixture of fat and water suggestive of brown adipose tissue. Warmer colors represent a higher proportion of adipocyte content, indicative of white adipose tissue. Note the particular variability present at one year of age in fat fraction in brown adipose tissues (white arrows, right panels).

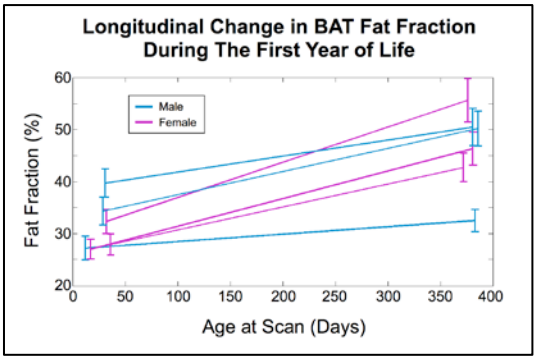


Figure 2. Longitudinal Change in BAT Fat Fraction. Longitudinal changes in fat fraction are presented for both male (blue) and female (magenta) participants. A pattern of increasing fat fraction was consistently observed across all participants.

#Abstract chosen for a travel award

Poster #39

Brown Fat Differentiation from Muscle Precursor Cells by novel Action of BMP6

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Brown adipose tissue (BAT) plays a pivotal role in promoting energy expenditure by the virtue of uncoupling protein-1 (UCP-1) that differentiates BAT from its energy storing white adipose tissue (WAT) counterpart. The clinical implication of "*classical*" BAT (that originates from Myf5 positive myoblastic lineage) or the "*beige*" fat activation in improving metabolic parameters is now becoming apparent. However, the inducers and endogenous molecular determinants that govern the lineage commitment and differentiation of *classical* BAT remain obscure. We report here that in the absence of any forced gene expression, stimulation with bone morphogenetic protein 6 (BMP6) induces brown fat differentiation from skeletal muscle precursor cells of murine and human origins. Through a comprehensive transcriptional profiling approach, we have discovered that two days of BMP6 stimulation in C2C12 myoblast cells is sufficient to induce genes characteristic of brown preadipocytes. This developmental switch is governed in part by newly identified regulators, Optineurin (Optn) and Cyclooxygenase-2 (Cox2), which have no previously known roles in *classical* brown adipogenesis. Furthermore, pathway analyses using the Causal Reasoning Engine (CRE) identified additional potential causal drivers of this BMP6 induced commitment switch. Subsequent analyses to decipher key pathway that facilitates terminal differentiation of these BMP6 primed cells identified a key role for Insulin Like Growth Factor-1 Receptor (IGF-1R). Collectively these data highlight a therapeutically innovative role for BMP6 by providing a means to enhance the amount of myogenic lineage derived brown fat.

Poster #40

Detection of Brown Adipose Tissue in Guinea Pig Fetuses using MRI

Kevin J Sinclair[1] BSc, Lanette J Friesen-Waldner[1] PhD, Colin M McCurdy[1] BSc, Curtis N Wiens[2] PhD, Trevor P Wade[1] PhD, Timothy RH Regnault[3,4] PhD, Charles A McKenzie[1,2] PhD
[1]Department of Medical Biophysics, [2]Department of Physics and Astronomy, [3]Department of Obstetrics and Gynaecology, [4]Department Physiology and Pharmacology, Western University

Introduction: Chemical shift-based water-fat separated magnetic resonance imaging (MRI) has been shown to be a powerful tool to image adipose tissue *in vivo* (1). Once the adipose tissue is separated, brown adipose tissue (BAT) can be distinguished from the white adipose tissue (WAT) due to differences in their fat composition. As BAT contains much smaller lipid droplets combined with an abundance of mitochondria and high vascularity, the proton density fat fraction (PDFF) is much smaller than in WAT (2). Observing brown fat during fetal development is of interest because altered brown fat content may be associated with later in life obesity (3).

In the current study, pregnant guinea pigs were scanned using the IDEAL water-fat separation method. Guinea pigs were chosen due to their similarity to humans in regard to adipose tissue development during fetal growth. PDFFs were obtained in fat pads within each fetus to determine the feasibility of observing brown fat using this technique.

Methods: Pregnant near term guinea pigs (N=10 with a total of 32 fetuses, scanned at ~ day 60 of ~ 68 day gestation term) were anaesthetized and scanned using a 3 Tesla MRI. IDEAL water and fat images (0.8mm isotropic resolution) were obtained using a 32 channel receive coil. PDFF values were obtained in both the interscapular and inguinal fat pads of each fetus, as well as in subcutaneous adipose tissue in the hind limb of the mother. Values were obtained by placing regions of interest (ROIs) in these fat pads on fat fraction images generated during post-processing.

Results and Discussion: PDFFs were significantly decreased compared to subcutaneous adipose tissue in the mother. Furthermore, PDFF values in the interscapular fat pad were slightly decreased when compared to those in the inguinal fat pads (76% compared to 85%). These results suggest that BAT and WAT are distinguishable in the fetus, and that brown fat exists in the inguinal fat pad, commonly taken to be WAT in adult rodents, as well.

Conclusion: IDEAL water-fat imaging can be used to detect brown fat within guinea pig fetuses displaying normal growth parameters. Using this technique, fetal brown fat development could be monitored. Translation to humans is possible with accelerated imaging techniques.

References:

1. Reeder SB, *et al*. *Magn Reson Med* 2005; 54(3): 636-644.

2. Hu HH, *et al*. *Magn Reson Med* 2010; 31(5): 1195-1202.
3. Sarr O, *et al*. *J Pregnancy* 2012; vol. 2012, Article ID 134758, 10 pages, 2012. doi:10.1155/2012/134758

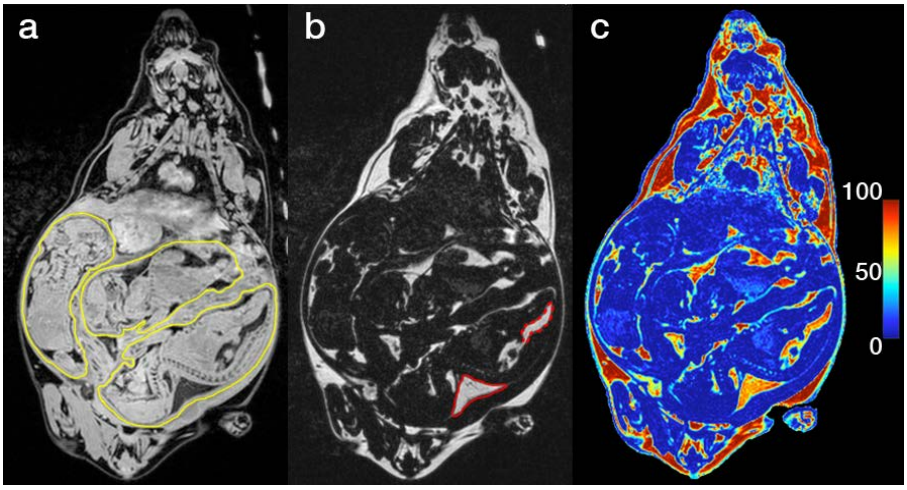

Figure 4: Coronal IDEAL water-fat separated images of a pregnant guinea pig. a) Coronal water only image with fetuses contoured in yellow, b) Fat only image with an intrascapular fat pad contoured in solid red and inguinal fat pad in dashed red, c) Fat fraction map with fat fraction denoted by colour bar

Poster #41

Human Brown Adipose Tissue Function during Muscular Work

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[1]Russian State University of Physical Education, Sports, Youth and Tourism (RSUPE), Moscow, Russia; [2]Institute of Developmental Physiology Russian Academy of Education, Moscow, Russia

The discovery of peptide "irisin" produced by muscles during exercise and promotes the transformation of white fat cells into "beige" fat cells [Boström et al., 2012], brings up the question about the role of human brown fat (BF) during physical work. It is known that physical activity stimulates precursor fat cell differentiation towards the "beige" [Spiegelman, 2013]. It means that some beneficial properties of BF, like the ability of BF cells to utilize lactate [Son'kin et al., 2010; De Matteis et al., 2012], might improve physical performance. This is confirmed by the presence of specific intracellular lactate transporter in mice BF [Iwanaga et al., 2009] and an abrupt increase in the activity of this transporter under the action of physical training in rats [De Matteis et al., 2012].

The aim of our study was to investigate whether human BF able to participate in the recycling of lactate and thus affect the physical ability.

Methods: Study was approved by RSUPESYT ethical committee and conducted in compliance with the principles of biomedical ethics by 73 young (20-32 years) healthy volunteers at different levels of motor training. Ramp test on a cycle ergometer or a treadmill under the control of heart rate and gas analysis were used to assess aerobic performance. To assess the body's response to cold exposure the dipping foot in the tub with ice water for 1 minute under the control of heart rate and gas analysis, and in some of the experiments - also myography and measuring of skin impedance were used. The samples of peripheral blood from the terminal phalanges of thumb for lactate measurement were taken during experiment. To detect activity of BF infrared thermal imager NEC TH 9100 in video mode, allowing to control the dynamic changes of the temperature of the selected skin area was used.

Results: Under normal room temperature and resting muscle lactate levels in peripheral blood varies randomly in the redistribution of 0.1-0.2 mmol / l. In 60 of 65 cases dipping the feet into ice water for 1 minute resulted in a decrease in blood lactate levels by 30-50%. After a single cooling leg reduction of lactate may take up to 10 minutes. Parallel to this oxygen consumption reflects non-shivering thermogenesis activity increases by approximately 50% compared to baseline. Signs of the contractile activation of thermogenesis were not registered. The temperature of the skin, which is the projection of BF infrared radiation, in the course of cold exposure increased by an average of 0,5-0,7°C and maintained at an elevated level for 5-7 minutes after it. The temperature of the skin or the other did not change or decreased. Vascular perfusion of the skin, measured

by the impedance, decreased. It reflects vasoconstriction in response to cooling. Thus, the specific activation of BF non-shivering thermogenesis caused by cooling the feet, leads to a rapid decline in blood lactate.

In another series of study (n=60) were compared the results of infrared dynamic thermography and functional changes in the process of the ramp test. Dynamic thermography allows visualizing the activation of subcutaneous BF during ramp test at the moment of anaerobic threshold, and especially at the time of completion of the work and the beginning of the recovery period. In both cases, the activation of the BF was combined with a dramatic increase in the level of blood lactate.
Our results indicate the active participation of the BF in the recycling of lactate during physical exercise. This is an important and entirely new fact in the exercise physiology, which has many important implications for the understanding of the physiological mechanisms of the health and efficiency as well as development of rational modes of training.

Poster #42

Autologous Matrix Assisted Cell Transplantation as a Strategy for the Expansion of Human BAT

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Brown adipose tissue (BAT) possesses the inherent ability to dissipate metabolic energy as heat in a process termed non-shivering thermogenesis and thus could lend itself to novel anti-obesity treatment approaches. Strategies for expanding BAT fall into two general categories, pharmaceutical/genetic intervention to trigger endogenous BAT differentiation pathways or *ex vivo* generation/expansion of brown fat followed by implantation. Since pharmacological activation of differentiation pathways that might drive a white adipose tissue (WAT) to BAT transition, or "browning" of WAT, runs the risks of affecting differentiation and function of other tissues and offers poor control of the location and extend of BAT expansion, we have focused on the latter approach. Our central hypothesis is that bioinspired hyaluronic acid hydrogels can be engineered to drive and support differentiation of white adipose tissue derived mesenchymal stem cells (ADMSCs) into functional BAT that can be used for autologous implantation to enhance metabolic rates and to promote weight loss and insulin sensitivity in obese subjects. Our general strategy has been to engineer BAT optimized hydrogels to support the differentiation of ADMSCs into BAT and the maintenance of functional BAT matrix assisted cell transplants (MACT) *in vivo*. Following the characterization of BAT-MACT persistence and metabolic function in recipient animals, we will utilize this system as a preclinical testbed for BAT-MACT based treatments of obesity related disorders. In preliminary studies we have been able to build a robust system that has allowed us to isolate WAT-derived MSCs, initiate differentiation under controlled conditions *in vitro*, incorporate the cells into bioinspired HyA hydrogels, and then complete BAT differentiation within the hydrogel niche *in vivo* in the context of normal or high fat diets. Preliminary results support the viability of this approach including data showing transduction-free BAT differentiation of ADMSCs, identification of BAT-optimized adhesion ligands, and the generation of a bioinspired HyA hydrogel niche for these cells that can support viable and metabolically active BAT tissues upon injection. Importantly, BAT-MACTs increase recipient metabolic rate, resistance to cold exposure, and improved glycemic control and body weight.

Poster #43

Non-Invasive Microwave Radiometry for Long Term Monitoring of hBAT Metabolism to Assess the Effect of Therapeutic Interventions

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Purpose: An inexpensive non-invasive approach is required to monitor the activity of human brown adipose tissue (hBAT) continuously over long time periods to assess the effect of therapeutic interventions. We present the design and experimental performance of a high-sensitivity long term stable radiometric thermometer system for non-invasive measurement of volume average temperature of hBAT regions located within 4cm of the tissue surface.

Methods: Thermodynamic analysis was performed to estimate the increase in hBAT temperature expected from increased metabolism. To quantify temperature changes of BAT depots at depth in the body, we constructed a 2.8cm diameter microwave radiometric sensor consisting of EMI-shielded log spiral microstrip antenna with directional on-axis beam profile for deep tissue penetration and integrated high gain multiband total power radiometer. Using electromagnetic simulation software, we optimized antenna design for collecting maximal signal from 2-6 cm^3 BAT volumes located up to 4cm deep in multilayer (skin-fat-muscle) tissue. Radiometer hardware was assembled with tuned high rejection EMI notch filters and multiple overlapping pass bands for discrimination of temperature-depth profile. Software was developed to provide drift compensation of amplifier gain that enables accurate and stable readings of deep tissue temperature over many hours. Performance of the radiometry system was assessed in terms of accuracy and stability of temperature readings in realistic multilayer phantom models with higher temperature BAT depots underlying lower temperature tissue layers.

Results: We report SAR patterns that characterize the frequency-dependent sensing region of the optimized receive antenna, and correlation of radiometric power measurements with deep tissue temperature that quantify instrument sensitivity. The data demonstrate our ability to accurately track temperature differences of <0.5°C (or less than 3-fold variation in metabolic activity) in 2-6cm^3 volumes of BAT located to 4cm depth in cooler tissue environment.

Conclusions: A non-invasive sensor consisting of miniature receive antenna and integral total power radiometer has demonstrated sufficient sensitivity to track clinically significant changes in temperature and metabolic activity of deep tissue targets underlying normothermic surface tissues over many hours. Combined with a wireless link to remote computer data logging, monitoring may be extended overnight to assess the long term effects of therapeutic interventions on hBAT activity. Clinical studies are

warranted to assess the accuracy of thermal profile estimation from multi-frequency radiometric measurements calibrated with thermodynamic simulations of patient-dependent BAT metabolism at depth.

Poster #44

Brown Adipose Tissue and Sleep Regulation*

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Introduction: The interaction between sleep and metabolism is well recognized. During sleep, characteristic changes in metabolic activities occur and experimental manipulation of metabolism affects sleep-wake cycles. Brown adipose tissue (BAT) plays a central role in the regulation of metabolism by determining energy expenditure and overall metabolic efficiency in the body. This is due to the action of uncoupling protein-1 (UCP-1), a protein that is found exclusively in the inner membrane of the mitochondria of brown adipocytes. We hypothesized that normal BAT function is necessary for optimal sleep. We tested our hypothesis by studying sleep in UCP-1 knockout (KO) mice that have non-functional BAT.

Methods and Results: Male UCP-1 KO and wild-type (WT) mice (n=8 per genotype) were used in the experiments. In the first set of experiment, spontaneous sleep-wake activity, body temperature and behavioral activity were recorded for 24 h on the first day. On the second day, mice were sleep deprived by gentle handling for the last 6 h of the light period and recovery sleep was recorded from the beginning of the dark period for 24 h. Baseline metabolism and feeding activity were recorded from separate groups of mice using indirect calorimetry. UCP-1 KO and control mice had similar diurnal rhythms of sleep with more non-rapid-eye movement sleep (NREMS) and rapid-eye movement sleep (REMS) during the light period than at night. UCP-1 KO mice had significantly more wakefulness during the light phase than controls (268 ± 16 min vs 224 ± 11min, respectively) and significantly more REMS during the dark phase (17 ± 3 min vs. 9 ± 1 min). The increases in the amounts of wake and REMS were due to the increased episode durations. UCP1 KO mice had more numerous and shorter NREMS episodes during the light phase than controls. Body temperature in UCP-1 KO mice was significantly lower during all experimental days and showed attenuated diurnal changes compared to control mice. UCP-1 KO mice had slightly lower heat production during the dark phase and lower respiratory quotients during the light phase than in WTs. There was no significant difference in the amount of food intake and in feeding activity between the two genotypes. Sleep deprivation induced rebound increase in NREMS and REMS in both control and KO mice, but the response of BAT deficient mice was attenuated. NREMS increased by 138 ± 13 min in control mice and 91 ± 13 min in the KOs, while the amount of REMS increase in KO mice was about 50 % of that of control mice (18 ± 3 min vs 33 ± 13 min, respectively). Thermosensitive sensory neurons arise from the BAT and project to brain regions known to be involved in sleep regulation. The aim of the second set of experiments was to test the hypothesis that sensory innervation of the BAT mediates the sleep-promoting effects of BAT activation. We tested spontaneous sleep, recovery sleep after sleep deprivation and the sleep-inducing effects of pharmacological stimulation of

brown adipocytes in mice with capsaicin-induced selective lesion of the sensory afferents arising from the interscapular BAT pad. Capsaicin was dissolved in an emulsion of 10% ethanol, 10% Tween and 80% isotonic NaCl. Mice were anesthetized and intrascapular BAT pads were injected with capsaicin or vehicle using 20 microinjections (1 µl/injection) spread across the entire BAT mass. After 3 weeks, mice were instrumented for sleep recordings. Following another 10 days of recovery, spontaneous sleep and sleep responses to 6 h sleep deprivation (gentle handling) were measured. In a separate experiment, mice were injected intraperitoneally with saline on the control day and with 0.2 mg/kg β3-adrenergic receptor agonist (CL-316,243) on the experimental day. Sleep, body temperature and motor activity was recorded for 24 h after each treatment. Under baseline conditions, intra-BAT capsaicin-treated mice showed similar sleep patterns to that of UCP-1 KO mice; NREMS decreased during the light and REMS increased during the dark period as compared to controls. NREMS and REMS rebounds after sleep deprivation were significantly attenuated in the BAT denervated mice. EEG slow-wave activity (SWA) was significantly increased for 12 h after sleep deprivation in control mice while this increase was significantly shorter-lasting in capsaicin-treated mice. CL-316,243 induced a robust sleep increase lasting for 12 h in the control mice. The responses were attenuated by ~50% in intra-BAT capsaicin-treated animals.

Conclusions: Present results support the hypothesis that BAT plays a role in maintaining normal sleep-wake activity and metabolism in mice and the sensory afferents from the BAT play a significant role in mediating sleep-promoting signals arising from the BAT.

*Abstract chosen for a Hot Topic talk

Poster #45

The Selective RORγ Inverse Agonist, SR1555, Improves Body Weight and Metabolic Readouts in Diet-Induced Obese Mice

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The retinoic acid receptor-related orphan receptors (RORs) are nuclear receptors that regulate multiple cellular processes, including metabolism, cellular differentiation, immunity and apoptosis. These receptors, which have constitutive and ligand-induced activity, function by binding to ROR response elements on target genes and facilitating recruitment of transcriptional machinery to elicit downstream effects. Recent reports have highlighted the potential role of RORs in human pathologies, such as autoimmune disorders and obesity, and novel RORα and RORγ synthetic ligands have been generated. Here, we explored the effect of a potent, RORγ-selective inverse agonist, SR1555, on body weight and metabolic readouts in diet-induced obese mice. We found that administration of SR1555 for three weeks significantly decreased body weight by 13% over vehicle. This decrease could, in part, be attributed to a 14% reduction in total food intake. In addition, we observed a 50% and 25% reduction in inguinal and gonadal fat pad weight, respectively, as well as a significant improvement in fed and fasted insulin levels, circulating cholesterol, triglycerides and high-density lipoprotein. In parallel with tracking changes in food intake, we were interested in the potential involvement of energy expenditure in mediating the effect of SR1555. For this, we examined inguinal fat pads for the expression of a key thermogenic marker, uncoupling protein 1 (UCP1), and found a seven-fold increase in UCP1 mRNA, as well as pockets of strong UCP1 staining by immunohistochemistry, warranting further investigation into functional readouts from this tissue. Taken in whole, our data suggests that modulation of RORγ activity with SR1555 significantly improves body weight, reduces adiposity and improves metabolic parameters, and highlights the potential for RORγ inverse agonists in the treatment of metabolic disease.

Poster #46

Human Brown Adipose Tissue Recruitment

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Introduction: Upon mild cold exposure people can increase their energy expenditure (EE) without shivering, which is defined as non-shivering thermogenesis (NST). When rodents are chronically exposed to cold shivering disappears, while EE remains elevated and BAT recruitment takes place. It was unknown if this could occur in humans well. However, recently we have shown that cold acclimation resulted in BAT recruitment in human adults, in parallel with an increased NST (1). In addition, we here present changes in glucose uptake rate in BAT in the supraclavicular region by exploring the methodology of 'fixed volume'.

Methods: In this study 17 young, healthy and lean humans participated, of which 9 females and 8 males. Cold acclimation was achieved by exposure to an ambient temperature of 15-16 °C for 10 consecutive days. Cold-induced BAT activity was measured using dynamic and static [^{18}F]FDG-PET/CT-imaging during maximal NST. An individualized cooling protocol was used, in which subjects were cooled down to a temperature just above the onset of shivering. Energy expenditure was measured by means of indirect calorimetry. These measurements were performed before and immediately after the cold acclimation period (1). Glucose uptake rates were calculated for total supraclavicular BAT region, additionally regions of similar volume (8 x 8 x 8 mm) were drawn on maximal SUV spots in the supraclavicular depot ('fixed volume' methodology).

Results: Static imaging revealed that BAT volume and activity (glucose uptake, expressed in standard uptake value; SUV) increased significantly upon cold acclimation (before: 665±451; after: 913±458 cc ($p<0.05$) and before: 2.4±0.7; after 2.8±0.5 SUV mean ($p<0.01$), respectively). Non-shivering thermogenesis increased from 10.8±7.5 to 17.8±11.1 % ($p<0.01$), and a significant relation was found between maximal SUV both before (R^2 0.55; $p<0.01$) and after the cold acclimation period (R^2 0.32; $p<0.05$) (1). Glucose uptake rate calculated for supraclavicular total BAT did not increase (6.9±3.0 before and 7.6±2.5 µmol/min/100g ($p>0.05$) afterwards). However the 'fixed volume' methodology revealed that glucose uptake rate did increase (before: 22.5±12.5; after: 33.5±16.8 µmol/min/100g ($p<0.05$)).

Conclusion: This study shows that BAT is recruited after cold acclimation in human adults. Both BAT activity and volume increased significantly. In parallel, non-shivering

thermogenesis increased. Additionally, BAT activity is related to NST before and after the cold acclimation period. This indicates that active BAT is a contributor to the increased energy expenditure upon cold exposure (1). An important outcome of the additional 'fixed volume' methodology is that cold acclimation increases glucose uptake rate.

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Poster #47

Contribution of Brown Adipose Tissue to the Regulation of Energy Expenditure in Healthy Men

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The metabolic activity of human brown adipose tissue (BAT) can be assessed by measuring FDG uptake by PET/CT. We have recruited more than 250 healthy volunteers during last 6 years, measured their BAT activity by FDG-PET/CT, and showed that 1) BAT activity is undetectably low under thermoneutral conditions, but increased greatly after 2-h exposure to cold at 19˚C (1), 2) BAT activity is positively and negatively correlated with cold-induced thermogenesis (CIT) and body fatness, respectively (1-4), and 3) repeated cold exposure increases BAT activity and CIT, and concomitantly decreases body fatness (4). These results suggest that BAT is involved in the regulation of whole-body energy expenditure (EE) and body fatness in humans.
The incidence and activity of human BAT show seasonal variations, being low in summer and high in winter (1). In the present study, we examined the relationship between BAT activity and EE, particularly focusing on their seasonal variations. Forty-five healthy male volunteers underwent FDG-PET/CT after 2-h cold exposure in winter to estimate their maximal BAT activity, were divided into high (n=30) and low (n=15) activity groups, and their body composition and EE were measured in winter and again in summer. Slight but significant differences in body fatness and fat-free mass were found between winter and summer. In winter, resting EE under a warm condition at 27˚C was 1,472 and 1,467 kcal/day in the high and low activity groups, respectively. After 2-h cold exposure at 19˚C, EE increased significantly in both the groups, but calculated CIT was greater in the high activity group (183 vs. 114 kcal/day). In summer, resting EE was slightly higher than that in winter both in the high and low activity groups, showing a positive correlation with fat-free mass. In contrast, EE did not increase even after cold exposure in either group. These results suggest a significant contribution of BAT to the regulation of whole-body EE, particularly to CIT in winter.

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