# Six Years with Adult Human Brown Adipose Tissue and Many More to Come

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Remarkably, it is still only 6 years ago that the existence of active brown adipose tissue in adult humans was first presented to the metabolic scientific community, based on data circulating quietly in the realms of radiology; and it is still only 4 years ago that a series of dedicated experimental studies convinced a broad scientific world that adult humans really possessed a "new" organ: brown adipose tissue. Given this short time, it is evident that the significance of this new organ is still far from settled.

Much effort is presently channeled into the questions: how much brown fat do we have – and how can we get more? There are several issues to be aware of in these contexts.

Firstly, there is no simple correlation between the amount of brown fat we have and its functional activity, definitely not if the amount is expressed as grams, but even if the capacity is expressed as amount of UCP1. One reason for this is that UCP1 cannot make heat in itself. There must be a commensurate increase in total mitochondrial oxidative capacity – as well as in blood supply and innervation to the tissue. Thus, just promoting UCP1 gene expression in molecular ways may be successful as such, but may lack thermogenic effects if no more substrate can be oxidised.

Another reason is that UCP1 is not leaky. Irrespective of how much UCP1 we have, its contribution to metabolism will be zero if it is not activated. Indeed, irrespective of how much potential thermogenic capacity we have, the brain will only request activation of the capacity it considers physiologically meaningful. Thus, the idea that merely increasing the amount of brown adipose tissue will counteract the development of obesity is not valid. This problem could be solved by stimulating the tissue pharmacologically, but then we lose the point of physiological control: in principle this would mean that the tissue would be active both in the warm and in the cold, and both when we overeat and when we undereat. Indeed, animal models of obesity demonstrate that it rather may be the ability to activate the tissue by metabolic clues that is missing, than the ability as such to recruit the tissue, e.g. through cold exposure.

Even the ways to measure brown adipose tissue recruitment are not trivial and should not rely on defining amount of brown fat in grams, nor on relative increases in UCP1 mRNA levels.

An important point will also be to identify the factors that govern the involution of brown adipose tissue that occurs with age. Clearly, these issues will be challenging for many years to come.

## Treating Metabolic Disease through Pharmacological Activation of Human BAT

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Cold-mediated activation of brown and white adipose tissue (BAT and WAT) has been effective in rodent models at increasing energy expenditure, insulin action, consumption of ingested glucose and triglycerides, and improvement of the metabolic profile. In humans, cold is currently the only effective way to stimulate BAT glucose uptake, and recent evidence has shown that chronic exposure can increase its activity. Though promising, this approach carries the risk of limited adherence, and its long-term safety and efficacy remain unknown. Understandably, the pharmacological activation of human BAT and WAT has been a goal for over three decades. Both broadly acting sympathomimetic drugs and more targeted β3-adrenregic receptor (AR) agonists have been used effectively in both cellular and animal models. In addition, multiple hormones have led to the browning of typically WAT depots, reinforcing the possibility that BAT energy expenditure could be activated indirectly through the central nervous system. Unfortunately, translation to humans has so far been unsuccessful. Sympathomimetic drugs such as ephedrine and isoproterenol produce marked cardiovascular stimulation with little influence on BAT. Over the past twenty years, several β3-AR agonists have reached the second phase of clinical trials only to fail because of suboptimal oral bioavailability, pharmacokinetics, or pharmacodynamics. However, none of these drugs were tested for their ability to stimulate human BAT, the fundamental proof-of-concept studies needed to clarify the utility of pharmacological BAT stimulation. With the recent ability to monitor human BAT metabolism using PET/CT, we are now on the threshold of a new era in BAT therapeutics.

## Fat Depots and Bone: The Role of Human BAT

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Excess white adipose tissue (WAT), primarily visceral adipose tissue, has been shown to be detrimental to bone in humans. In contrast, recent studies have indicated a positive link between brown adipose tissue (BAT) and bone mass, bone structure and muscle. BAT can be imaged using 18F-fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (FDG-PET/CT) and studies using FDG-PET/CT have shown that BAT can persist in humans well beyond infancy and is found primarily in the supraclavicular and paravertebral regions. In addition to the "classic" brown adiopocytes attributed to derivation from a myogenic cell lineage, brown adiopocytes can be induced after alterations in temperature or noradrenergic pathways to appear in WAT anatomic sites termed "beige" or "brite". In adults, these beige/brite adiopocytes, more akin to white adipocyte precursors, may represent most of what is imaged by FDG-PET/CT- particularly when cold-induced. Clinical studies have focused on predictive factors of BAT including age, weight, temperature, insulin resistance, use of beta-blockers and the impact of BAT on overall energy expenditure—all factors which impact bone biology. BAT is lower in obese humans and decrease during aging. Aging is a time when thermoregulation is altered with deficiencies in thermoreception, thermogenesis, and conservation. Aging is also a time when bone loss becomes apparent and BAT may be a contributing factor to age-related bone loss. In a study using FDG-PET/CT in young non-obese women, including women with anorexia nervosa (AN), women recovered from AN (AN-R), and normal weight controls, only one of five women with AN demonstrated cold-activated BAT, which may be due to impaired BAT thermogenesis in AN, whereas in the AN-R group two of five and in the HC group four of five subjects were BAT positive. Women with positive BAT on PET/CT had higher bone mineral density (BMD) by DXA, higher T3, and lower preadipocyte factor-1 (Pref-1), a regulator of mesenchymal stem cell differentiation, than BAT-negative women, independent of disease status, suggesting that BAT may be involved in the regulation of stem cell differentiation into the bone lineage. Furthermore, IGF binding protein 2 (IGFBP-2), which binds IGF-1 in the circulation and has been reported to inhibit bone formation in humans, was inversely associated with BAT and BMD, suggesting that IGFBP-2 may serve as a regulator of BAT-mediated osteoblastogenesis. Subsequent studies in children and adults with a history of successfully treated malignancy, have confirmed a positive associations between BAT and bone. BAT volume was a positive predictor of femoral cross-sectional area and cortical bone area, which was in-part mediated by muscle. Improving our understanding of the interactions between inducible BAT, energy expenditure and bone formation and the underlying hormone regulators of these processes, could lead to effective therapies to prevent bone loss.

## Brown and Beige Fat: Basic Biology and Novel Therapeutic Opportunities

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Brown and beige fat can prevent or reverse obesity and diabetes in rodents by increasing energy expenditure and the reduction of blood and tissue lipids. The is a great deal of interest in activating brown fat in humans as a novel approach to metabolic diseases. However, the separate roles of brown and beige fat in mammalian physiology have been very difficult to separate. We have recently made a murine model that has a severe reduction in the thermogenic function of beige fat cells, while leaving the classical brown fat largely intact. These Adipo-PRDM16 KO mice develop a mild obesity, accompanied by hepatic steatosis and hepatic insulin resistance. Furthermore, such mice show a "visceralization" of the gene expression patterns in their subcutaneous fat, suggesting that PRDM16 and beige fat play a key role in the functional differences between subcutaneous and visceral adipose tissues. We have also launched studies to find new polypeptides that are involved in activating and augmenting brown and beige fat thermogenesis. We have found that PGC1 expression in muscle control the expression of Fndc5, a cell membrane protein. Fndc5 is proteolytically cleaved to give rise to a new secreted protein of 112 amino acids that we have called irisin. Irisin circulates in both mouse and man, and even mild elevations of irisin activate the browning of white fat, causing increased energy expenditure and reducing obesity. We have also found that the PGC1 coactivators in muscle stimulate the secretion of a neurotrophic factor called meteorin-like. Elevation of meteorin-like levels in the blood of mice activates both beige and brown fat thermogenesis, partly through effects on the immune cells in the fat depots. Finally, prospects for novel therapies involving beige fat, irisin, meteorin-like and other novel myokines will be discussed.

## Does Cold-activated Brown Fat Contribute Significantly to Daily Energy Expenditure?

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**Objectives:** Contrary to previously held belief that brown adipose tissue (BAT) disappears shortly after birth, FDG PET has shown its presence in adult humans. This has generated a lot of interest in its role in daily energy expenditure (EE) and possible weight management through its activation. The objective of our study was to determine, using dynamic oxygen-15 ($^{15}O$) PET imaging, the extent of thermogenesis (i.,e., EE), contributed by activated BAT in adults during cold stress and to establish the relationship between BAT oxidative metabolism and FDG tracer uptake.

**Methods:** Twenty five adult normal subjects (15F/10M, 30±7 years) underwent triple oxygen scans ($H_2{}^{15}O$, $C^{15}O$, $^{15}O_2$) as well as measurements of EE (kcal/day) at both rest and following exposure to mild cold (60$^{o}$F) using indirect calorimetry. Subjects were divided into two groups (BAT+ and BAT-) based on the presence or absence of FDG uptake (SUV > 2.0) in cervical–supraclavicular BAT. Blood flow and oxygen extraction fraction (OEF) was calculated from dynamic PET scans at the location of BAT, muscle and white adipose tissue (WAT). The metabolic rate of oxygen ($MRO_2$) in BAT was determined and used to calculate the contribution of activated BAT to daily EE.

**Results:** The median mass of activated BAT in the BAT+ group (8F/1M, age 30±6y) was 52.4g (range 14 to 68g) and was 1.7g (range 0 to 6.3g) in the BAT- group (7M/9F, age 31±8). Corresponding maximal SUV values were significantly higher in the BAT+ as compared to the BAT- group (7.4±3.7 vs. 1.9±0.9; p=0.03). Blood flow values in BAT were significantly higher in the BAT+ group as compared to the BAT- group (13.1±4.4 vs. 5.7±1.1 ml/100g/min, p=0.03), but were similar in WAT (4.1±1.6 vs. 4.2±1.8 ml/100g/min) and muscle (3.7±0.8 vs. 3.3±1.2 ml/100g/min). Moreover, OEF in BAT was similar in the two groups (0.56±0.18 in BAT+ vs. 0.46±0.19 in BAT-, p = 0.39). Calculated $MRO_2$ values in BAT increased from 0.95±0.74 to 1.62±0.82 ml/100g/min in the BAT+ group and were significantly higher than those determined in the BAT- group (0.43±0.27 vs. 0.56±0.24; p = 0.67). The increase in EE associated with BAT oxidative metabolism was highly variable in the BAT+ group, with an average of 5.5±6.4 kcal/day (range 0.57–15.3 kcal/day).

**Conclusions:** BAT thermogenesis in humans accounts for a very small fraction of daily EE during moderate cold stress, even in subjects with relatively large BAT depots. As a result, it is unlikely that cold-activated BAT can contribute significantly to weight management in obese individuals.

## Novel Approaches to Investigating Fatty Acid Metabolism in Human BAT

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The role of brown adipose tissue (BAT) plays in human metabolism is expanding by the findings that BAT, in healthy adults, is functionally active (Virtanen et al. 2009, van Marken Lichtenbelt et al. 2009, Cypess et al. 2009, Saito et al 2009). Thermogenesis is regarded as one of the most important functions of BAT, but based on *in vitro* data other functions exist. These functions include the clearance of triglycerides from the circulation (Bartelt et al. 2011).

BAT has triglyceride stores deposited in small lipid droplets, intracellularly, and fatty acids are rapidly delivered from these droplets for fuel in activated mitochondria. In healthy subjects, approximately one-third of these lipid stores, during cold, could be mobilized (Ouellet et al 2012). The lipid storage may be estimated using Hounsfield units (HU), which are derived from CT images based on tissue densities (water content). This capacity may be used for the determination of active vs. nonactive BAT, such as in cold and in warm (Hu & Gilsanz 2011, Ouellet et al. 2012, Hu et al. 2013).

Another option for the noninvasive estimation of BAT lipid storage is MRI. MR imaging is based on the spin effect of water molecules, thus the tissue content of water may be used for the tissue differentiation. Basal MRI is not very applicable for the distinction of active BAT from nonactive BAT but MR spectroscopy ($^{1}$H-MRS) may be used for the determination of fat content of the tissue. The higher triglyceride content of BAT, determined by MR imaging, the tissue may be nonactive BAT (which resembles WAT).

Traditional anatomical imaging methodologies (CT and $^{1}$H-MRS) may be used for the estimation of tissue triglyceride content and its change during cold exposure. However, synchronized with intracellular triglyceride consumption, tissue uptake of fatty acids from the circulation can be ascertained. This process fulfills the diminishing storage of triglycerides and supplies mitochondria with excess fuel. Fatty acid uptake in tissue may be measured with PET using the fatty acid analogue, [$^{18}$F]FTHA, which is a long-chain synthetic fatty acid PET tracer (DeGrado et al. 1991, Ebert et al. 1994). [$^{18}$F]FTHA is taken up from the circulation similar to fatty acids but its intracellular metabolism is halted after β-oxidation in mitochondria. Trapping of [$^{18}$F]FTHA gives a precise measure of the influx rate in the tissue but the rate must be multiplied with plasma FFA concentration in order to calculate the FA utilization rate. By using this [$^{18}$F]FTHA tracer during cold and ambient temperatures, new mechanisms by which BAT utilizes fatty acids in various metabolic states can be determined.

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Financial support for the studies of BAT fatty acid metabolism has been received from the Academy of Finland, EU FP7 DIABAT, Paulo Foundation, The Diabetes Research Foundation, Finnish Cultural Foundation, The Finnish Medical Foundation

## The Norepinephrine Transporter: A Novel Target for Imaging Brown Adipose Tissue

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**Background:** Obesity is characterized by a relative impairment of energy expenditure associated with adaptive thermogenesis. Studies in animals have shown that adaptive thermogenesis involves activation of brown adipose tissue (BAT), a tissue previously thought to completely regress in adult humans. Positron emission tomography (PET) scans, verified with tissue biopsy, support the continued existence of BAT in adults. Understanding the extent to which BAT in adult humans plays a role in energy balance and obesity has been limited by the current detection methodology using FDG-PET, which requires cold stimulation. The purpose of this study is to test a new, mechanistically driven approach for imaging BAT in humans that capitalizes on the fact that BAT is strongly innervated and regulated by the sympathetic nervous system (SNS) machinery. More specifically, we propose to image the norepinephrine recycling component, designated the norepinephrine transporter (NET). We hypothesize that the NET-PET imaging approach using (*S,S*)-[$^{11}$C]O-methylreboxetine) ([$^{11}$C]MRB), a highly selective NET ligand, will provide a non-stimulated target for BAT that is proportional to BAT mass. In this talk, our evaluation of using [$^{11}$C]MRB as a tool for NET-PET imaging both preclinical in rats and clinical in humans will be discussed.

**Methods:**

**Preclinical *ex vivo* and *in vivo* evaluation in rats of the specificity of [$^{11}$C]MRB labeling for BAT**: PET images of male Sprague-Dawley rats with [$^{18}$F]FDG and [$^{11}$C]MRB were compared. Relative [$^{18}$F]FDG or [$^{11}$C]MRB retention at 20, 40 and 60 min post-injection was quantified on awake rats after exposing to cold (4$^{o}$C for 4h) or remaining at room temperature (RT). Rats pretreated with unlabeled MRB or nisoxetine 30 min before [$^{11}$C]MRB injection were also assessed. The [$^{11}$C]MRB metabolite profile in BAT was also evaluated.

**Clinical evaluation in humans of the specificity of [$^{11}$C]MRB labeling for BAT:** Five healthy, Caucasian subjects (4 male, 1 female; age 23.6±1.8 years; BMI 22.4±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 RT and mild cold stimulated conditions (Cool vest, with 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. Mean standardized uptake value (SUV) for FDG and [$^{11}$C]MRB, and distribution volume ratio

(DVR) for [$^{11}$C]MRB were estimated via MRTM2 using occipital brain area as the reference region. A ratio of BAT-DVR to muscle-DVR (BAT/muscle) were also computed and compared.

**Results:**

**Preclinical evaluation:** PET imaging demonstrated intense [$^{11}$C]MRB uptake (SUV of 2.9 to 3.3) in the interscapular BAT of both RT and cold-exposed rats, and this uptake was significantly diminished by pretreatment with unlabeled MRB. In contrast, [$^{18}$F]FDG in BAT was only detected in rats treated with cold. *Ex vivo* results were concordant with the imaging findings; *i.e.*, the uptake of [$^{11}$C]MRB in BAT was 3 times higher than that of [$^{18}$F]FDG at RT ($P=0.009$), and the significant cold-stimulated uptake in BAT with [$^{18}$F]FDG (10-fold, $P=0.001$) was not observed with [$^{11}$C]MRB ($P=0.082$). Furthermore, there were no correlations between FDG uptake (either at RT or cold) with BAT mass; whereas there was a good correlation between MRB uptake at RT with BAT mass ($R^2=0.945$). HPLC analysis revealed 94-99% of total radioactivity in BAT represented unchanged [$^{11}$C]MRB.

**Clinical evaluation:** [$^{18}$F]FDG uptake in BAT was observed in all subjects under mild cold conditions, but not at RT (SUV: RT 0.69±0.08 vs. cold 3.36±2.00). However, [$^{11}$C]MRB uptake occurred in BAT in the same anatomic locations as seen in the [$^{18}$F]FDG scans under both RT and cold conditions, and the difference of [$^{11}$C]MRB uptake between RT and cold was not significant [BAT-DVR: RT 0.91±0.21 vs. cold 1.12±0.35; or DVR (BAT/muscle): RT 2.16±0.66 vs. cold 2.36±0.74].

**Conclusion:** Both of our preliminary preclinical and clinical imaging studies have shown that [$^{11}$C]MRB can efficiently label BAT in rats and humans at both RT and mild cold conditions. In contrast, [$^{18}$F]FDG labeling of BAT occurred only under mild cold conditions. These preliminary data suggest that [$^{11}$C]MRB-PET may provide a non-stimulated target for imaging BAT in humans, that appears to be quantitatively proportional to BAT mass.

**Supported by NIH/NIDDK.**

## Insight into the Functional Organization of Human BAT

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Brown adipose tissue thermogenesis plays a role in energy metabolism and affords an attractive target for drugs and methods intended to control weight gain. Yet, the functional organization of BAT and the kinetics of thermogenesis in the intact animal and in humans remains to be resolved. To address this gap, we carried out first in-man mapping of the distribution of the mitochondria membrane potential (ΔΨ)-targeting voltage sensor 18F-FBnTP in BAT before and during cold stimulation in healthy volunteers, using dynamic PET. ΔΨ is an effective parameter for (1) assessing BAT mass at rest, (2) predicting potential thermogenesis capacity and (3) quantifying kinetic variables of evoked thermogenesis.

**Methods and Results:** In 11 subjects, 90-min dynamic FBnTP PET of the upper shoulder area was acquired before and during mild cold stimulation. In 7 of 11 subjects, cold FDG PET was carried out within a week after FBnTP PET. Supraclavicular BAT (sBAT) depots were identified in 8/11 subjects. FBnTP reached plateau concentration within a few minutes, which was maintained for the duration of basal condition, with sBAT-to-background > 3:1. In contrast, mitochondrial uncoupling induced by mild cold stimulation resulted in biphasic washout of FBnTP from sBAT (27% to 58% of basal activity). Time to onset of regional FBnTP washout response varied widely across sBAT from few to several tens of minutes. Consequently, activation of BAT, in the spatial and quantitative domains, was not an all-or-none but a time-dependent progressive process. BAT regions responsive to 60 min cold stimulation constituted 34% to 62% of total BAT mass measured at basal condition. The spatial distribution of FBnTP at rest correlated well with cold FDG PET/CT. In some subjects, FDG underestimated, and in one subject, missed BAT, detected by rest-FBnTP. Intriguingly, sBAT regions missed by FDG demonstrated no FBnTP washout during cold stimulation (i.e., non-responsive), a result supporting the functional significance of FBnTP washout response. Some sBAT regions demonstrated cold-FDG, but not rest-FBnTP uptake - most likely, representing sBAT regions uncoupled at ambient temperature.

**Conclusions:** Targeting ΔΨ with FBnTP PET provides insight into the functional organization of human BAT. FBnTP capacity to distinguish BAT mass (at rest) and activity (at cold stress) allowed to identify and quantify key variables of the thermogenic function (regional response latency, kinetics and magnitude) by contrasting with basal activity, within-subject. FBnTP PET provides evidence for the first time that BAT is a heterogenous mosaic of foci of variable response thresholds. Combining FBnPT with FDG provides a comprehensive map of BAT, upon which tonically active BAT depots may be identified. This study also provides evidence for FDG PET underestimation or missing existing BAT depots, that this may occur due to lack of evoked thermogenesis, and suggests that prolongation of cold stimulation may reduce FDG misdiagnosis. FBnTP-FDG capacity to dissect thermogenic components of BAT provides quantitative measures for better understanding and assessing BAT relationship with body weight, as well as unravel potential targets for means intended to potentiate mitochondrial biogenesis and bioenergetics.

## 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$^{o}$ lactate/bicarbonate, 3$^{o}$ 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.

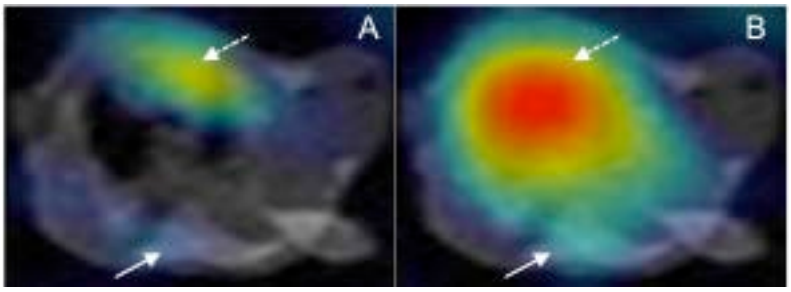


**Figure:** Overlay of $^{13}C$-bicarbonate images 20 seconds post injection, on $T_1$ weighted axial images pre (A) and post (B) stimulation of metabolism by isoproterenol. (Solid arrows: subscapular fat pad, dashed arrows: heart.) The increased signal post stimulation indicates increased aerobic metabolism.

**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.

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*Abstract chosen for a Hot Topic talk

## Deciphering Brown Adipogenesis from a Progenitor and Inductive Signaling Perspective

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Brown adipose tissue, owing to its inherent capacity to convert excess food energy into thermal energy, has become a focus of biomedical research as a means to counter the spread of obesity. Rodent data suggest the existence of two distinct types of brown fat: the classical BAT, which derives from a myf5-lineage and is anatomically located in the interscapular region of mice, and the recruitable BAT (also known as beige or brite) that emerges in white fat from a non-myf5 lineage. While BAT has been studied for a long time in rodent cell models, there is an urgent need for human brown fat progenitor models to study physiological characteristics of human brown fat and ultimately to develop new therapeutic approaches for the treatment of obesity. Recently, we established pairs of immortalized human brown and white fat progenitors from anatomically defined human neck fat. The immortalized cells retained morphological and differentiation characteristics of primary cells. We have previously demonstrated that the developmental factor bone morphogenetic protein 7 (BMP7) serves as a strong promoter of brown adipogenesis. We have characterized two different strains of brown fat progenitors derived from two subjects. Interestingly, these two cell strains exhibited distinct features representing progenitors of brown and beige adipocytes. Upon induction of differentiation, cell strain-A expressed very high levels of UCP1, but did not respond to inducers of brown adipogenesis to further stimulate the thermogenic program. By contrast, cell strain-B expressed a relatively lower level of UCP1, but both UCP1 mRNA and protein could be robustly induced when treated with BMP7 prior to induction. Importantly, differentiated cells from strain-B responded to brown fat inducers and agents mimicking adrenergic stimulation to boost UCP1 expression. These data suggest the existence of at least two distinct types of human brown fat progenitors. The established cells provide valuable human cellular models for advancing our understanding of human brown adipogenesis.

## Pharmacological Approaches to Induce Brown and Beige Fat Cell Development

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Current evidence indicates that there are two types of UCP1-positive thermogenic adipocytes arising from distinct developmental lineages. A classical form of brown adipocytes is mainly found in the interscapular brown adipose tissue (BAT) deposits and develops from muscle lineage. In contrast, an inducible form, termed “beige” or “brite” cells, develops within subcutaneous white adipose tissue (WAT) in response to several external cues including chronic cold exposure. We and other groups have recently shown that PRDM16 and EHMT1 are both required for the formation of classical brown adipocytes and inducible beige cells. To pharmacologically activate the developmental pathways of brown and beige cells, we have developed new genetic tools to visualize and quantify the UCP1-positive cells *in vivo*. This allowed us to perform high-throughput screenings and identify chemical inducers of UCP1 and cellular respiration. Ongoing studies will be discussed in details.

## The Role of FGF21 in Brown Fat

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Fibroblast growth factor 21 (FGF21) is a multifunctional metabolic regulator which has potential applications in the treatment of diseases such as diabetes, obesity, and fatty liver. When administered in animals FGF21 has a plethora of metabolic activities. Recently published data suggest that FGF21 variant induces several beneficial effects in diabetic humans demonstrating that targeting FGF21 pathway is a viable avenue for metabolic drug discovery. Nevertheless, to date the mechanism and site of FGF21 action is not fully understood. Recent studies suggesting some of FGF21 effects are mediated indirectly and occur via modulation of secretion from adipose tissue of other hormonal factors such as adiponectin and leptin. In addition, FGF21 transgenic animals are characterized by enriched pool of brown adipocytes, and when FGF21 is administered to rodents it induces adipose tissue browning. In order to evaluate if FGF21 effects on brown adipocytes or browning of adipose tissue underlie FGF21 pharmacology in mice and if yes which particular facets of FGF21 mechanism we tested FGF21 in mice with total body ablation of UCP1, a BAT biomarker. Some preliminary results stemming from this study will be discussed at this talk.

## 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

## Brown Adipose Tissue Quantification Using Magnetic Resonance Imaging

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**Background:** Scientific interest in brown adipose tissue (BAT) has dramatically increased in recent years. Human BAT was previously believed to only exist during fetal growth, infancy, childhood and early adolescence. One of the most exciting recent discoveries is the prevalence of BAT in adult humans, primarily revealed by $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) positron emission tomography (PET) imaging. The existence of BAT in adult humans is motivating investigations of BAT's role in numerous aspects of physiology, including metabolism and energy balance, obesity, and musculoskeletal development. Enhancing the volume or activity of BAT could improve the clinical management of many metabolic disorders, but such studies will require a safe, non-invasive method for BAT quantification that can be applied repeatedly in a broad range of human subjects. Reliable identification and spatial mapping methods that can distinguish BAT from white adipose tissue (WAT) would provide a powerful tool to study BAT. Unlike PET, magnetic resonance imaging (MRI) is free of ionizing radiation, and the ability to identify and quantify BAT using MRI will have a substantial positive impact on the pursuit of new avenues of research and potential treatments involving BAT.

**Purpose:** The overall purpose of this presentation is to describe methods for volumetric quantification and metabolic activation detection of BAT using MRI. Part one of the presentation will cover the recent work showing how BAT can be detected and quantified separately from WAT and non-adipose background tissues using MRI approaches based on the different MR properties of water and lipid protons. This presentation will discuss in detail both past and recent research efforts to characterize BAT morphology and metabolic activity with proton spectroscopy and chemical-shift-based fat-water MRI techniques. Specifically, MRI measurements of fat signal fraction (FSF) will be described, and published evidence of the effectiveness of FSF to detect BAT in animal and human subjects will be presented. The second part of the presentation will conclude with an outlook on future research and comments on other potential MRI-based strategies for characterizing BAT such as measurements of longitudinal (T1), transverse (T2) and phenomenological transverse (T2*) relaxation rates, water and lipid apparent diffusion coefficients, blood perfusion, and thermometry based on proton resonance frequency (PRF) or T1 changes.

**Conclusion:** MRI-based volumetric quantification and metabolic activation detection of BAT in humans will enable studies of BAT's connection to metabolism, endocrinology, body composition and obesity across a wide range of subjects and may lead to clinical studies of the positive effects of BAT activity for counteracting metabolic disorders.

## Brown Adipose Tissue Perfusion: From Mice to Men

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When activated by cold and sympathetic nervous system stimulation, brown adipose tissue (BAT) increases energy expenditure by using fatty acids and glucose to produce heat. Brown adipose tissue is a highly vascularized tissue and the activation of BAT is accompanied by a further increase in blood flow. The increase in blood flow is thought to deliver additional substrates and oxygen to the BAT and is necessary for BAT-induced thermogenesis. Until now, $^{18}$F-fluorodeoxyglucose positron emission tomography coupled with CT scan ($^{18}$F-FDG PET-CT) is the only technology available to detect human BAT *in vivo*. However, these machines are rare, the experiments are expensive, and because of radiation exposure it is not possible to do repeated studies in an individual under various conditions. Taking advantage of the high vascularity of BAT, we are developing the use of contrast-enhanced ultrasound (CEU) to detect and monitor functional BAT in mice and humans. Contrast-enhanced ultrasound involves the intravenous infusion of echogenic microbubbles during the ultrasound procedure, allowing the estimation of tissue blood flow. We report that CEU detects baseline blood flow in mice BAT and the BAT blood flow increase induced by an infusion of norepinephrine. BAT perfusion is decreased in obese dB/dB mice and in UCP1-deficient mice. In young and lean human volunteers, CEU detects and estimates blood flow in the supraclavicular adipose tissue. The estimated adipose blood flow increases after cold exposure. In 8 volunteers, the supraclavicular adipose blood flow estimated by CEU correlates with the supraclavicular BAT $^{18}$F-FDG uptake estimated by $^{18}$F-FDG PET-CT. In summary, contrast-enhanced ultrasound estimates BAT blood flow, and changes in flow induced by BAT activation, in mice and in humans. This noninvasive, non-irradiating, widely available, and affordable technique can be used to detect BAT and its activation in mice and potentially in humans.

## Detecting BAT Mass and Thermogenic Activity by Using Nonlinear $^{1}$H MRI and Hyperpolarized $^{129}$Xe Gas MRI

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In recent years, the discovery of active brown fat in adult humans (*1, 2*) and the observation that BAT activity is inversely correlated with BMI have presented the possibility that this tissue be used as a possible target for obesity treatment(*3*). To this end, researchers are investigating interventions that decrease body weight in overweight and obese subjects by increasing energy expenditure through BAT volume and/or activity modulation(*1*).
Despite the importance of this tissue, most of our knowledge on BAT stems from animal studies, and very little is known about this tissue in humans. There exists little data to assess the incidence of this tissue in the adult human population, to clarify the observed inverse association between BAT activity and BMI, and to determine whether overweight and obese subjects in which BAT mass and activity is currently not detected really lack BAT and/or thermogenic activity. The gold standard to detect human BAT, $^{18F}$DG-PET/CT, is highly non-specific, especially in overweight and obese subjects. BAT thermogenesis is based largely on the oxidation of fatty acids released from intracellular triglyceride stores rather than glucose (*4*). Only when the intracellular lipid storage is depleted, as it may be in lean individuals, does the tissue start to rely on circulating fatty acids or glucose to sustain thermogenic activity(*5, 6*).

MRI is very attractive for studying BAT since it is non-invasive, does not deliver mutagenic radiation, and has no limitations in imaging penetration depth. Fat fraction measurements by $^{1}$H are indeed gaining attention as an alternative way to detect BAT independently of its metabolic activity(*7*).However, in adult humans this tissue is present only in scattered amounts, and partial volume effects caused by different types of cells in a single voxels (for example WAT, BAT, and muscle) can lead to both false positive and false negative findings.

To overcome this limitation we use nonlinear $^{1}$H MR techniques to detect both BAT mass and function. The critical advantage of $^{1}$H MR is our ability to produce background free BAT maps. We can probe fat fraction at a much smaller scale than that currently accessible with conventional MR techniques, and thereby eliminate confounding partial volume effects. We will present our small animal and human work, underling the major advantages and limitations of these nonlinear 1H MR techniques.

In an effort to develop a radiation free method that can be used to non-invasively monitor BAT mass and thermogenic function in both lean and obese subjects, we started to investigate the use of hyperpolarized xenon gas MRI to detect BAT. This technique, currently used to detect lung ventilation function in humans, is ideally suited to detect BAT mass for several reasons. First, the gas is an ideal perfusion tracer: after $^{129}$Xe inhalation, the amount of the gas found in any organ is directly proportional to tissue perfusion. This means that large local changes in blood flow such as those that occur upon stimulation of BAT thermogenic activity (*8*) can easily

be detected. Because of the high solubility of this gas in lipids(*9*), circulating xenon naturally diffuses into the highly perfused BAT, enabling adequate SNR for imaging, while the absence of intrinsic xenon in tissues allows for the acquisition of background free BAT maps. Secondly, the broad chemical shift range of xenon allows for a clear differentiation between xenon dissolved in blood from xenon dissolved in cell cytoplasm versus xenon dissolved in fat molecules, providing a means to directly and specifically probe BAT tissue hydration. Lastly and more interestingly, the linear temperature dependence of the chemical shift of xenon dissolved in lipids allows for a direct measurement of BAT cell temperature and tissue thermogenic activity. We will show our MR imaging, spectroscopy, and thermometry experiments with HP xenon gas in lean, obese and UCP1 knock out animals. We will show that HP xenon gas can produce background free maps of BAT in just few minutes in lean as well obese animals, directly monitor BAT cell temperature with an accuracy better than 0.1C, and detect differences in BAT thermogenic capacity between animals. The possibility to detect BAT mass and its thermogenic activity in human subjects fills the much needed gap in detection methods to allow us to specifically monitor BAT metabolism and investigate this tissue in overweight and obese individuals.


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## Passive and Active Microwave Radiometry for the Non-Invasive Detection of Human Brown Fat

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Medical microwave radiometry is attractive for research on human brown fat because it is completely safe, noninvasive, fast, and the instrumentation is portable. Passive medical microwave radiometers use small receiving antennas that are placed on the skin of the subject to passively receive the internally generated thermal microwave noise leaving the skin at the locations where the antennas contact the skin. Such passive measurements make it possible to detect differences in subsurface temperatures at various antenna locations from depth much greater than measured by infrared thermometers. Experimental results of measurements with passive microwave radiometers on human volunteers aimed at detecting the presence of metabolically active human brown fat will be presented at a poster session. Active medical microwave radiometers inject safe levels of amplified microwave thermal noise into the tissues of the subject by means of small transmitting antennas placed on the skins of the subjects and measure either transmitted or reflected amplified noise power with small receiving antennas also placed on the skins of the subjects. From such active measurements it is possible to detect differences in the amount of blood perfusion between various receive and transmit antenna locations. Experimental results obtained with active microwave radiometers aimed at detecting changes in blood perfusion in humans and in tissue equivalent phantoms will be presented. Future microwave radiometers designed specifically for research on brown fat will be described including wearable radiometers for continuously monitoring the activity of brown fat and radiometers for scanning large areas of the body.

## 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

## Effect of Ambient Temperature on Human Brown Fat Activity and Recruitment

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Animal studies clearly indicate the flexibility of brown adipose tissue (BAT) in response to environmental temperature. BAT is activated by acute cold exposure and secondly the amount of BAT depends on long-term environmental temperature, i.e. recruitment of BAT in the cold and deterioration in warm conditions.

In humans functional cold activated BAT has been shown by imaging glucose uptake by means of positron emission tomography (PET) combined with computed tomography (CT), using a glucose tracer (FDG) ($^{18}$F-FDG-PET/CT). Studies on biopsy material indicate that humans have both classical brown adipocytes and so called brite/beige adipocytes, each from different cell lineages and with different cellular characteristics.

Only recently studies have paid attention on BAT recruitment in humans. Several lines of evidence now exist that indeed apart from activation, BAT can be recruited. First of all older studies indicate that exposure to cold outdoor temperatures result in relatively large amounts of BAT tissue.
The first physiological proof of BAT recruitment was a study on weight reduction. The reason for BAT recruitment caused by weight reduction, was probably the increased cold sense due to the negative energy balance during the period of weight loss (starvation effect).

This year two cold acclimation studies have been published that clearly show that the nonshivering thermogenesis capacity increases along with an increase in BAT activity and volume. The results of these studies will be discussed in depth and linked to our current knowledge on the dynamics and localization of classical BAT and brite/beige adipose tissue/cells.

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## Exploring the Roles of Brown Fat in Children

Vicente Gilsanz, MD, PhD, Children's Hospital Los Angeles, Los Angeles, CA

In this presentation, we will highlight areas of progress in pediatric brown adipose tissue (BAT) research over the past 5 years, including the general acceptance that this tissue is more prevalent in children than adults and in infants and teenagers than in prepubertal children. Available data in pediatric patients indicates that children with BAT have significantly greater muscle volume than those without identifiable BAT. Moreover, a positive association between muscle volume and the amount of BAT has been reported in both boys and girls. A recent longitudinal study also provides strong evidence in support of an inverse association between BAT activity and white adipose tissue accumulation, most strikingly in the intra-abdominal depot. Although the lack of techniques to measure BAT has limited our understanding of its relevance to human physiology, advances in magnetic resonance imaging techniques now allow for the quantification of BAT, even in healthy infants.

## Signal Transduction Pathways Converging to Promote 'Brown(ing)' of Adipocytes

Sheila Collins, Ph.D., Marica Bordicchia, Ph.D., Zuzana Kováčova, Ph.D., Dianxin Liu, Ph.D., Richard Pratley, M.D. Diabetes and Obesity Research Center, Sanford-Burnham Medical Research Institute, Orlando, FL and Translational Research Institute for Diabetes and Metabolism, Orlando, FL

Catecholamines and the β-adrenergic receptors (βARs) are well known activators of brown fat thermogenesis and promoting the expansion of 'brown' adipocytes within white adipose tissue (WAT) depots (BRITE). Activation of the βARs not only increases lipolysis but activates a transcriptional program for this 'brown' adipocyte expansion. Recently other hormones and signaling pathways have been described that can influence early developmental differentiation toward the brown or white adipocyte lineage, or from a more therapeutic perspective be able to function in a manner akin to catecholamines. The cardiac natriuretic peptides (NPs), ANP and BNP, were shown to stimulate lipolysis in human adipocytes. They can also activate brown fat thermogenesis and the program of brown adipocyte expansion within WAT, in the same manner as βAR agonists. NPs activate a transmembrane receptor (NPRA) that produces cGMP. A clearance receptor (NPRC) removes NPs from circulation. The relative levels of NPRA vs NPRC dictate the ability and strength of the response to NP signaling. Mice lacking NPRC have less body fat and become resistant to diet-induced obesity and insulin-resistance. In clinical studies obesity and insulin resistance are associated with a significantly reduced ratio of NPRA/NPRC In adipose tissue. It has been proposed that the increased levels of NPRC in adipose tissue of the obese may contribute to the reduced circulating levels of NPs and hypertension that accompanies obesity. Assessing the ability of NPs and NP analogues in the clinic will further our understanding of their role in adipose 'browning' and body composition.

## 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

## 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

## 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.

*Abstract chosen for a Hot Topic talk

## Hope for a Therapy Based on Thermogenic Drugs and BAT

Eric Ravussin, Ph.D.

Pennington Biomedical Research Center, Louisiana State University System

The last few years have heralded a renaissance in the study of brown adipose tissue (BAT) and in particular, the exploitation of BAT as a means to increase energy expenditure and dissipate excess calories. This notion was first suggested in the 1970s from studies showing that rodents fed cafeteria-diets were able to increase both the amount and activity of BAT. It is now well-accepted that brown adipose tissue is highly prevalent in adult humans as detected by studies using $^{18}$F-FDG PET/CT scanning. However there are limitations to this technique including its poor reproducibility, inability to detect difference between BAT presence and activity and the need for radiation precluding its use in most healthy subjects and children. I will provide in my presentation a historical overview of brown adipose tissue research, summarize recent developments in the field and concludes with a discussion of the questions which must be addressed before considering the stimulation of BAT as an anti-obesity therapy. Also, I propose an integrated physiological approach for understanding BAT at the whole body level and emphasize that approaches for stimulating BAT will likely be opposed by mechanisms which act to increase energy intake. The promise of BAT as an obesity therapy is still at a preliminary stage. Realistically, if one was able to activate BAT in humans (discrete BAT and “beigeing” of adipose) sufficiently to dissipate calories, it would almost certainly need to be combined with other therapies which target long-term energy balance signals including improved satiety.

## Does hBAT Play a Role in the Characteristics of Non-Shivering Thermogenesis?

Kong Y Chen, Robert J Brychta, Sarah Smyth, Jacob Hattenbach, Suzanne McGehee, Laura Musse, Paul Lee, Marc Reitman, Francesco Celi

Despite of early pioneer work by Du Bois and Hardy and others from the 1930-1960's, surprisingly little is understood about how the body regulates heat production (energy expenditure, EE) in response to subtle changes in environmental temperature. Only the recent realization of brown adipose tissue in adult humans (hBAT) rejuvenated the interests to quantify its role in thermogenisis. It is plausible that the mechanisms governing heat production contribute to regulation of body weight and thus may be contributing to the current obesity epidemic. The goal of our current research focuses to unfold the characteristics of the EE to environmental temperature response in humans, specifically relating to the non-shivering thermogenesis (NST). Using a custom-designed metabolic chamber (whole-room indirect calorimeter), we are mapping the resting EE response to environmental temperatures ranging from 16-31°C (12 randomized temperature points) while rigorously controlling for clothing, diet, physical activity. We are also measuring core and skin temperatures, heart rate and its variability, muscle activity, thermal comfort and hunger, hBAT by 18-FDG PET/CT, and hormone levels. We lowered room temperatures to each individual's shivering threshold. Using the dynamic resting EE and environmental temperature data, we constructed the NST response curve based on the first principle and derived slope and lower critical temperature parameters. In our first study cohort (of 12 healthy young lean Caucasian males), there is a wide range of NST capacity (5-32%) and amount of hBAT (10.2-163.3ml). We will explore the potential relationships between the amount (Volume) and activity (mean SUV) of hBAT and the characteristics of NST (capacity - %REE, slope, lower critical temperature, and shivering threshold). We will lay out future study directions and discuss limitations of our studies.

## Insights from Understanding the CNS Pathways Regulating the Sympathetic Outflow to BAT

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From mouse to man, BAT thermogenesis is an important component of the homeostatic repertoire to maintain body temperature during the challenge of a low environmental temperature and to combat infection by elevating body temperature during fever. The sympathetic outflow to BAT is a key determinant of the intensity of BAT thermogenesis and is regulated by neural networks in the CNS that respond not only to skin thermoreceptor inputs, but also to a variety of central neurochemical and peripheral sensory signals related to metabolic and energy status. Augmenting the level of BAT energy consumption could be effective in reducing adipose energy stores and in improving glucose utilization. Conversely, inhibiting BAT thermogenesis could be a useful strategy to lower brain and core body temperatures and thereby reduce metabolism in pathologies of compromised oxygen delivery (e.g. stroke, myocardial infarction), uncontrolled fever, and possibly cachexia. Improved understanding of the functional organization and neurochemical influences within the CNS networks that regulate the level of BAT sympathetic nerve discharge will suggest novel approaches for pharmacological manipulation of BAT calorie consumption and heat production.

## 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

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

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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