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Modulation of adipose tissue thermogenesis as a method for increasing energy expenditure ☆

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ABSTRACT

There is a renewed interest in the role of adipose tissue in energy utilization and thermogenesis and its potential application in the treatment of metabolic disorders such as obesity and diabetes. The last few years have seen the identification of brown adipose tissue capable of metabolic activation in adult humans, the possibility of recruiting 'beige' adipocytes to increase energy expenditure, and the implication of molecules such as FGF21 and irisin in inducing increases in energy expenditure in adipose tissue. The translation of these findings into human trials to deliver safe, efficacious medicines remains a challenge.

Adipose tissue and energy balance: The fundamental cause of obesity is a chronic imbalance between energy intake and energy expenditure, leading to storage of excess energy in adipose tissue. As the well-recognized growing incidence of obesity and related co-morbidities continues, the identification of new treatment paradigms to supplement the known benefits of exercise and a controlled diet in establishing a negative energy balance and hence driving weight loss becomes more urgent. To date, the successes of pharmacologically driven weight loss approaches have been limited by safety concerns and moderate efficacy. Alternatives to pharmacological intervention or lifestyle alterations such as bariatric surgery have been successful, but due to the invasive nature these options demand careful consideration.[1] The determination that adult humans possess significant deposits of adipose tissue in the supraclavicular and neck regions that are capable of metabolic activation upon cold exposure has lead to the reinvigoration of work to understand the possible relevance of adipose tissue mediated energy disposal in man and reignited the possibility of increasing energy expenditure via pharmacological intervention in adipose tissue.

Adipose tissue itself plays a central role in the regulation of energy storage and expenditure. White adipose tissue (WAT) is the primary energy storage compartment within mammals. In white adipose tissue, energy is stored in the form of triglycerides in a single large lipid droplet, which occupies the majority of the volume of the white adipocyte, resulting in their characteristic, peripherally located nucleus. White adipocytes possess relatively few mitochondria. In addition to its role in energy storage, WAT constitutes a key endocrine organ that is involved in the regulation of appetite and satiety via the secreted hormones leptin and adiponectin, which have been the focus of intense research for a number of years.[2,3]

In contrast to WAT, brown adipose tissue (BAT) is specialized in energy expenditure. Brown adipocytes are multilocular cells, containing many small lipid droplets and a relatively high number of mitochondria. Brown adipocytes are unique in their expression of *SLC25A7*, the gene encoding the mitochondrial uncoupling protein 1 (UCP1).[4] Embedded in the inner mitochondrial membrane, UCP1 acts as a proton conduit from the intermembrane space to the mitochondrial matrix. Activation of UCP1 leads to the energy generated by the electron transport chain and stored in the pH and electrochemical gradient across the inner mitochondrial membrane to be dissipated as heat, rather than used to fuel ATP generation by ATP-synthase.

BAT thermogenesis was for many years thought to lack physiological relevance in adult humans, as the large interscapular BAT depot used by newborns to regulate body temperature disappears in the first few decades of life. However, the existence of other brown fat depots in adults has been known for decades,[5] and the possibility of a relevant role for BAT in adult human metabolism was reinvigorated in 2009 with the determination that adult humans possess significant deposits of brown fat in the supraclavicular and neck regions capable of metabolic activation upon cold exposure.[6–8] Adipocytes taken from these areas of metabolically activatable fat were shown to be immunoreactive for UCP1.[9] The

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metabolic activity of BAT, defined by functional increases in ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake observed by PET/CT upon cold exposure, has a negative correlation to BMI.[6,8,10–12]

The successful use of 2,4-dinitrophenol, a protonophore mitochondrial uncoupler, as a means of increasing basal metabolic rate *in humans* constitutes a strong argument for the ability of a mitochondrial 'energy-wasting' approach to drive weight loss;[13,14] however, the use of 2,4-dinitrophenol has been associated with a number of hyperthermia related fatalities.[15] As 2,4-dinitrophenol does not rely on an endogenous protein target for efficacy, its uncoupling effect is not limited to a specific tissue depot. In contrast, brown adipose dependent thermogenesis is limited to a single tissue and relies on the activity of UCP1, potentially reducing the maximum inducible level of mitochondrial uncoupling and mitigating any risks associated with excess energy release.

The amount of energy that can be dissipated via existing BAT in a relevant patient population and the viability of using existing BAT as a clinical target to drive weight loss has yet to be established. Based on cold-induced increases in ^{18}F-FDG uptake, and assuming that glucose uptake accounts for only 10% of the total metabolic activity of brown adipose, Virtanen, et al. report an increase in energy expenditure in a representative patient sufficient to dissipate the energy equivalent of 4.1 kg of adipose tissue over one year if continuously fully activated.[7] Taking the energy content of white adipose to be 9 kcal/g, this equates to an increase in energy expenditure of approximately 1.5 kcal/day/gram of BAT. Using similar assumptions, an average increase in energy expenditure of approximately 0.8 kcal/day/gram of BAT can be derived from increases in ^{18}F-FDG uptake reported by Ouellet, et al.[16] A confounding factor is the large interindividual variation in the amount of activatable BAT reported in imaging studies. Some subjects have active BAT at room temperature, others do not show increases in ^{18}F-FDG uptake upon cold stimulation.

Lineage of thermogenic adipocytes It has been demonstrated in mice that brown and white adipocytes have separate developmental origins.[17,18] Murine brown adipocytes from the interscapular BAT depot were shown to derive from precursors positive for the myogenic transcription factor *myf5;* thus, 'classical' brown adipocytes share a common lineage with skeletal muscle. It has been established the transcriptional regulator PRDM16 controls a bidirectional cell fate switch between skeletal myoblasts and brown fat cells. The loss of PRDM16 from brown fat precursors initiates the loss of brown fat phenotype and promotes differentiation into myocytes. In the complementary experiment, induced expression of PRDM16 in myoblasts initiates their differentiation into brown fat cells.[17] WAT does not share this lineage.

A third type of adipocyte has been identified, referred to as 'beige',[19] 'brite',[20] or 'recruitable'[21] adipocytes. Unlike 'classical' brown adipocytes, murine beige adipocytes are from a non *myf5* positive lineage and have a low basal expression of UCP1.[19] However, like 'classical' brown fat they respond to stimulation by cAMP or β-adrenergic receptor agonists by increasing UCP1 expression and respiration rate. Beige adipocytes have a gene expression pattern that is distinct from either white or brown adipocytes. In particular, *CD137* and *TMEM26* act as markers for this cell type.

The presence of the full range of adipocytes from white to beige to brown has recently been demonstrated in man. Human adipose tissue isolated from the supraclavicular region of adult humans was stained with antibodies recognizing CD137 and TMEM26, demonstrating the presence of beige markers. The interscapular adipose that acts as a thermogenic organ in human infants has been confirmed to consist of 'classical' brown adipose tissue.[22] In adults, the complete range of human adipocytes has been shown to exist along a steep gradient in the fat deposits of the neck, from adipocytes the near the skin that expresses almost no UCP1, to fat closest to the center of the neck with high UCP1 expression. Depots of potential beige adipocytes exist between the two areas.[23]

With the mixed composition of human adipose tissues and the current varied nomenclature used in the field, it should not be forgotten that the ultimate functional marker for thermogenic adipose tissue is the presence of UCP1 protein. It has been noted that increases in *UCP1* expression in brite/beige fat on the order of 200-fold still lead to total UCP1 levels that are orders of magnitude lower than those found in classical BAT, due to the very low levels of UCP1 originally present in the recruitable cells.[24] This serves to reiterate the current functional uncertainty in targeting UCP1 mediated energy 'wasting'. Is there sufficient BAT present in the relevant patient populations to make a metabolic difference upon activation? Can it be safely activated? How large is the available pool of functionally recruitable beige adipocytes? Will recruitment of the beige adipocyte pool provide a level of energy expenditure comparable to or greater than that provided by the already existing BAT?

Pharmacological stimulation of thermogenesis: Human BAT is a highly innervated and vascularized tissue[9] and the activation of human BAT metabolic activity following exposure to cold is, at least in part, controlled by sympathetic nervous system (SNS) regulated secretion of norepinephrine. Administration of propranolol, a β-adrenergic receptor antagonist, reduces ^{18}F-FDG uptake in BAT depots in humans.[25,26] The mechanistic linkage between β-adrenergic stimulation and BAT thermogenesis may lie in the supply of free fatty acids released via lipolysis upon β-adrenergic activation. Free fatty acids can undergo β-oxidation, serving as the fuel for thermogenesis, and also play a role in the activation of UCP1. It has been shown that UCP1-mediated proton transport in mitoplasts isolated from mouse BAT is consistent with a fatty acid shuttling mechanism, wherein protons are transported in concert with the movement of a fatty acid within UCP1.[27]

Attempts to recapitulate the SNS-mediated stimulation of BAT activity via systemic administration of β-adrenergic agonists has met with mixed results. The efficacy of β-adrenergic agonists in increasing energy expenditure is not in question; however, the role of BAT in this response is not firmly established. Increased uptake of ^{18}F-FDG was not observed in areas traditionally thought to be BAT depots after systemic administration of the sympathomimetics ephedrine and isoprenaline at doses that cause increases in total body energy expenditure comparable to cold exposure.[28,29] Higher doses of ephedrine have been reported to activate BAT ^{18}F-FDG uptake in lean, but not obese, subjects.[30] The total increase in energy expenditure caused by administration of ephedrine was similar between lean subjects who possessed activatable BAT and obese subjects that did not, suggesting that the contribution of BAT to the ephedrine derived increase in energy expenditure in this case was minimal. It should be noted that ephedrine induces significant increases in heart rate and blood pressure at all doses administered in the above studies, including those insufficient to stimulate BAT ^{18}F-FDG uptake.

Several selective agonists of the adipocyte specific β_3-adrenergic receptor have been developed, Figure 1, and advanced to the clinic as part of efforts to activate adipose tissue metabolism while avoiding general adrenergic stimulation; however these efforts have led to largely disappointing clinical results.[31–33] Increases in energy expenditure were observed on administration of the selective β_3-adrenergic agonists L-796568[34] and TAK-677,[33] but a sustained increase capable of leading to meaningful weight loss was not observed in either case. A third agonist, CL-316243, did not induce significant changes in energy expenditure.[31] Although not targeted at activation of BAT activity *per se*, and without any evidence of a direct effect on BAT activity, the lack of sustained clinical efficacy reported with the selective β_3-adrenergic receptor agonists suggests that obtaining metabolically meaningful levels of BAT