Weight Loss at Altitude-The Untold Story: It's Cold Up Here!

By

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This is to acknowledge that Dr. Palmer has not disclosed any financial interests or other relationships with commercial concerns related directly to this program. Dr. Palmer will not be discussing off-label uses in his presentation.

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Purpose: Currently, there is a significant percentage of the population who are or will be classified as obese, necessitating novel strategies to facilitate sustainable weight loss. Reductions in basal metabolic rate occur in the face of weight loss, and pose formidable barriers to individuals attempting to sustain meaningful weight reductions. This grand rounds discusses the mechanisms by which non-shivering thermogenesis may provide insight into metabolic pathways that can become druggable targets to facilitate sustainable weight loss. Specifically highlighted is fact that non-shivering thermogenesis results in activation and expansion of brown and beige adipose tissues as well as activates pathways in skeletal muscle which increase metabolic flux and activity of muscle fibers through futile calcium cycling across the endoplasmic reticulum all facilitating an increase in metabolism. Finally, the protocol highlights the fact there are sexual dimorphisms with respect to these metabolic processes in keeping with the National Institutes of Health mandate of treating sex as a biologic variable.

Educational Objectives:

At the conclusion of this lecture, the listener should be able to:
1. identify the metabolic changes that occur with weight reduction leading to increased skeletal muscle efficiency
2. list the metabolic pathways that characterize non-shivering thermogenesis in brown adipose tissue and skeletal muscle
3. identify the features that give rise to a sexual dimorphism in non-shivering thermogenesis

The contents of this protocol are currently in press:

Palmer BF, Clegg DJ. Non-Shivering Thermogenesis as a Mechanism to Facilitate Sustainable Weight Loss. Obesity Reviews. 2017 (in press)

Introduction

Weight gain occurs when energy intake from food exceeds energy expenditure. By contrast, restricting caloric intake to a value below that needed for daily energy expenditure will result in weight loss. Similarly, physical activity or an increase in metabolic rate/energy expenditure that exceeds caloric intake will also result in weight loss. Many subjects find it extremely difficult to initiate, maintain, and sustain weight loss due to the requirements of consistent caloric deprivation. Currently, the most efficacious and sustainable weight loss regime is bariatric surgery, and while this procedure is an effective weight loss method, it is invasive and can be associated with morbidity and mortality. Current pharmacologic interventions are limited by availability of effective drugs to provide for and sustain weight loss. Given the increasing prevalence of obesity worldwide and its associated adverse effects on quality of life and average life expectancy, there is a need for more effective weight loss strategies.

When energy intake is sufficiently reduced so that body weight begins to decline, there is a physiological adaptation in basal metabolic rate which impedes further and sustained weight loss. To overcome this response, additional reductions in energy intake or increases in energy expenditure are required. Reductions in basal metabolic rate in the face of weight loss, pose a formidable barrier to individuals attempting meaningful weight reduction. Given the body's natural resistance to weight loss, and the fact that body weight homeostasis is comprised of two major elements, caloric intake and energy expenditure, the most effective weight loss strategy is to suppress appetite and at the same time maintain or even increase energy expenditure. This grand rounds will review and focus on a novel non-pharmacologic approach to maintaining basal metabolic rate in the setting of weight loss through the process of energy wasting through 'non-shivering thermogenesis'.

Weight Loss and Decreased Energy Expenditure

Skeletal muscle mass is the primary determinant of basal metabolic rate. Skeletal muscle mass is relatively fixed, but with the addition of weight-bearing exercise, muscle mass can be increased. Many weight reduction programs therefore combine reductions in caloric intake with a rigorous weight-bearing exercise program in an attempt to increase muscle mass and maintain or sustain basal metabolic rate in the face of caloric deficits to enhance long term weight loss success. This strategy was employed in 16 participants of a nationally televised weight loss competition, "The Biggest Loser". Over the course of 30 weeks the participants lost 57.6 kg or 40% of their initial body weight. Weight loss was comprised mostly of fat/adiposity while because of the incorporation of physical activity, fat free muscle mass was mostly maintained. Surprisingly, despite the relative preservation of fat free mass, resting metabolic rate decreased by 789 kcal/d, an amount greater than what could be accounted for by the change in body weight and composition. Participants were then re-evaluated 6 years later, and most subjects regained a significant amount of weight, consisting predominately of adipose tissue, resulting in their mean weight being only 11.9 % below their starting weight. In the face of their significant weight regain, their resting metabolic rate remained at a value not appreciably different from the end of the competition at 30 weeks, being approximately 500 kcal/d lower than predicted based on body composition and age. These important and relatively surprising results highlight two main points: 1) 6 years after significant weight loss and regain, their resting energy expenditure was lower than individuals who were weight matched but, whom had never lost weight, and 2) despite weighing more than they did at the end of the weight loss challenge, their metabolic rate did not change – supporting a concept which will be discussed later in this paper, and that is that adipose tissues are considered

metabolically 'inert' and contribute little to overall basal metabolic rate. Furthermore, these findings demonstrate that there is a prodigious metabolic adaptation put into place to defend against further reductions in body weight following weight loss. These metabolic adaptations appear to be sustainable, and not easily reset, making long term weight loss a persistent struggle. Reductions in basal metabolic rate associated with weight loss is thought to be an adaptive process designed to preserve body weight as if the body were in a state of famine. Evidence suggests this process is brought about by an increase in 'metabolic efficiency' such that fewer calories are required to maintain body weight when compared to an individual who has not undergone weight loss.

One explanation for these metabolic adaptations is that there appears to be a localized decline in energy expenditure accompanying weight loss which is associated with a concept of 'increased skeletal muscle efficiency'. To further elucidate this, in one study the researchers followed two cohorts of individuals following a 2-week period of weight stabilization, one group of otherwise healthy subjects were fed a calorie restricted liquid formula diet to achieve a 10% reduction in body weight and were then compared to subjects whose body weight increased by 10% following ad libitum food consumption. The researchers found that total energy expenditure decreased as predicted in the weight loss group. For the cohort that gained weight, there was an increase in energy expenditure by approximately 15% above baseline and both changes on energy expenditure were predictive based on changes in body composition. The component of energy expenditure that was primarily affected was non-resting energy expenditure defined as the energy required to perform physical activity (Figure 1). In this study, when the investigators went on to measure whole body indirect calorimetry during cycle ergometry as well as examined isolated gastrocnemius muscle ATP flux with magnetic resonance spectroscopy, they showed the variance in non-resting energy expenditure with weight perturbation could be accounted for by changes in skeletal muscle work efficiency; specifically, they found decreased efficiency with weight gain and increased efficiency with weight loss. In the presence of weight loss, increased muscle efficiency would oppose the maintenance of the reduced body weight since ATP and caloric cost for muscle contraction is reduced.

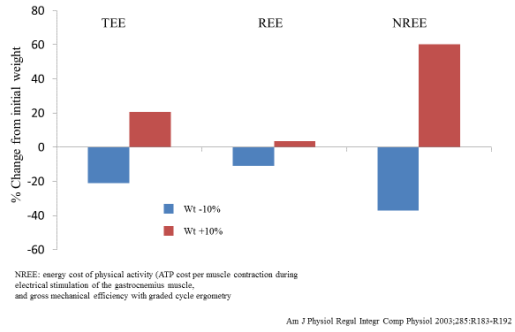

Figure 1: Energy expenditure with changes in body weight

Measurement of the respiratory quotient indicates changes in muscle efficiency may be related to alterations in fuel preference use within skeletal muscle as well as the muscle fiber type. In the weight loss group, there was a significant reduction in the respiratory quotient suggesting a change in fuel preference to be that of fatty acids consistent with a shift to greater use of slower twitch muscle fibers. This fiber type consumes fewer calories and generates less power per muscle contraction and derives a greater proportion of energy from the oxidation of fatty acids in comparison to fast twitch fibers. By contrast, the respiratory quotient increased in the weight gain group suggesting a greater reliance on glycolytic fast twitch muscle fibers which are less mechanically efficient.

In support of these findings, biochemical analysis of needle biopsy specimens taken from the vastus lateralis muscle of individuals support predictions that alterations in body weight are associated with changes in the respiratory quotient. Following weight loss, there was significant

declines in glycolytic enzyme activity. In particular, the ratio of glycolytic to oxidative enzyme activity was decreased. The ratio of glycolytic (phosphofructokinase) to oxidative (cytochrome c oxidase COX) enzyme activities correlated indirectly with increased skeletal muscle work efficiency and utilization of free fatty acids as a primary fuel source. In addition, there was a significant increase in relative gene expression of the more efficient myosin heavy chain I (MHC I) mRNA and sarcoplasmic reticulum Ca^{2+}-ATPase SERCA2a. These changes, along with increases in the ratio of MHC I/MHC II and MHC IIa/MHC IIx expression are all consistent with a lower potential to oxidize glucose relative to fatty acids providing a mechanism for prolonged aerobic contractions at a significantly lower energy cost. Importantly, the resulting reduction in energy expenditure is of a sufficient magnitude to contribute to the high recidivism, or weight re-gain, in those individuals who attempt to lose sustainable body weight.

Strategies to Maintain Energy Expenditure in the Setting of Weight Loss

Changes in sympathetic nervous system tone and thyroid hormone metabolism accompany the increase in muscle efficiency that develops with weight loss. Specifically, there are data to suggest there are reductions in sympathetic nervous system tone as measured by heart rate analysis and urinary catecholamine excretion. Additionally, circulating concentrations of thyroxine (T4) and triiodothyronine (T3) are significantly reduced from baseline following weight loss. These changes in sympathetic tone and bioactive thyroid hormones are reminiscent of that observed in leptin deficient states. Leptin is a hormone released from adipose tissues, and when there is a reduction in adipose tissue mass, there is also a reduction in circulating leptin levels. Therefore, it is consistent that following weight loss, there is a reduction in leptin which impinge on circulating thyroid levels. Normalization of leptin levels in leptin-deficient rodents increases energy expenditure, sympathetic nervous system tone, and normalizes thyroid function. The connections between leptin levels, energy expenditure, and thyroid hormones raises the possibility that if alterations in leptin levels sets the feedback loops in motion, restoration of exogenous leptin levels in the face of weight loss may be a useful strategy to maintain energy expenditure, and this would then facilitate sustainable weight maintenance in the presence of weight loss.

In fact, the administration of leptin to obese or lean subjects at their usual body weight has much less effect on energy homeostasis in comparison to subjects who have lost weight and are relatively hypoleptinemic in comparison to their original baseline leptin levels. This increase in leptin responsiveness was demonstrated in subjects fed a liquid formula diet to induce a 10% reduction in body weight and then given twice-daily subcutaneous injections of leptin designed to restore circulating leptin levels present prior to the weight loss or a placebo. Leptin administration to individuals who had lost weight reversed the decline in energy expenditure and increased skeletal muscle work efficiency to values present prior to the weight loss. This increase in energy expenditure resulted in a further decrease in fat mass in comparison to the control group ingesting an isocaloric diet. These data imply that leptin is a key regulator of metabolic efficiency in the presence of weight loss, and that restoration of leptin to the leptin replete state blunts the shift toward fatty acid utilization as a fuel source. Additionally, exogenous leptin blunts the decrease in respiratory quotient and glycolytic enzyme activity and reduces the increase in the relative gene expression of the more efficient myosin heavy chain I (MHC I) mRNA.

The effects of leptin to maintain energy expenditure in the presence of weight loss may either be through restoration of sympathetic nervous system tone and/or circulating concentrations of T3 and T4 to pre-weight loss levels. Leptin acts in the central nervous system where it has previously been demonstrated that direct application of leptin in rodent models increases

sympathetic tone and induces skeletal muscle thermogenesis. Leptin directly binds to receptors in the arcuate nucleus within the central nervous system and increases the expression of proopiomelanocortin (POMC) and its cleavage product α-melanocyte stimulating hormone (MSH) which in turn binds to the melanocortin-4 receptor (MCR4) and melanocortin-3 receptor. It is through this pathway that leptin brings about a suppressive effect on appetite and an increase in sympathetic nerve activity resulting in increased energy expenditure. Disruption in this pathway leads to obesity. For example, rare defects in the gene encoding POMC lead to hyperphagia and early-onset obesity while heterozygous mutations in the MCR4 are the most common cause of monogenic obesity.

In highlighting the important role of the direct and indirect effects of leptin in the central nervous system, there was a recent report of 2 patients with POMC deficiency, who were treated with the MCR4 agonist setmelanotide resulting in reduced hunger and substantial weight loss (51 kg over 48 weeks in one patient and 20.5 kg after 12 weeks in the second). In diet induced obese Rhesus Macaques, parenteral administration of a MCR4 agonist reduced body weight over an 8-week period and increased energy expenditure by 14%. In a randomized, double-blind, placebo controlled, crossover study of 12 obese human subjects, administration of a MCR4 agonist as a continuous infusion over 72 hours increased resting energy expenditure by 6.4%. All of these findings support the notion that there are both direct and indirect effects of leptin in the central nervous system and that stimulation of the MCR4 receptor suppresses appetite and increases energy expenditure and therefore provides an attractive adjunct therapeutic strategy to aid in weight reduction.

Additionally, leptin may act on the leptin receptors present on skeletal muscle providing a direct pathway for leptin to stimulate thermogenesis and increase energy expenditure. Leptin has been shown to directly stimulate thermogenesis in skeletal muscle in part through substrate cycling between de novo lipogenesis and lipid oxidation. Leptin stimulates fatty acid oxidation and glucose uptake through both direct effects on skeletal muscle as well as through the aforementioned hypothalamic sympathetic nervous system. In skeletal muscle, leptin fuels the increase in fatty acid oxidation through translocation of CD36 into the plasma membrane to facilitate fatty acid uptake. In a mouse model lacking UCP1 to prevent thermogenic activity in brown adipose tissue (BAT), survival is not possible at temperatures <12C unless leptin is administered. The rescue effect of leptin is associated with increases in the circulating levels of thyroid hormone and increased skeletal muscle SERCA 2a. Whether the increased capacity for skeletal muscle thermogenesis is a direct effect of leptin or mediated through T3 is not known.

Non-Pharmacologic Approaches to Increase Basal Metabolic Rate

Hypobaric Hypoxia

While pharmacologic approaches such as administration of exogenous leptin or a MCR agonists show promise, the requirement for parenteral administration and the potential for untoward side effects makes a non-pharmacologic approach a much more desirable way to facilitate sustainable weight loss. A discussion of non-pharmacologic approaches to increase energy expenditure will be provided, the first being ascent to altitude.

Exposure to hypobaric hypoxia is associated with an increase in basal metabolic rate, while at the same time there is a dose dependent suppression of appetite, making this an ideal yet less than feasible method of weight loss. Obese subjects taken to altitude and residing in an environmentally controlled station for seven days lose weight due to reductions in appetite with concomitant increases in basal metabolic rate (25). In support of this observation, population

studies show an inverse relationship between residing at high altitude and prevalence of obesity, as well diabetes.

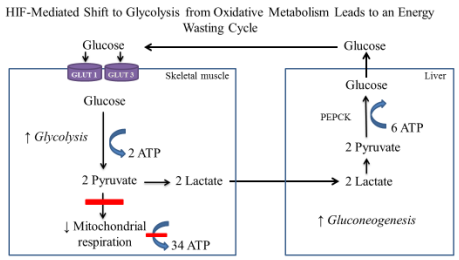

Figure 2: Metabolic changes leading to energy wasting at altitude

As recently reviewed, activation of hypoxia inducible factor (HIF) may play a major role in this response (Figure 2). A decrease in oxygen availability activates HIF which in turn leads to a reduction in appetite and an increase in energy expenditure by upregulating POMC and leptin through a direct effect on gene transcription. Activation of HIF also leads to a shift in metabolism away from oxidative phosphorylation to glycolysis. While this shift is an adaptive response to limited oxygen availability, since metabolism of glucose via glycolysis requires less oxygen as compared to oxidative phosphorylation, energetically it is less efficient and creates an energy wasting state which contributes to the increase in energy expenditure.

Shivering Thermogenesis

Another method to achieve an increase in energy expenditure is exposure to cold. Physiologic and metabolic pathways are activated by cold exposure designed to produce heat and maintain the body temperature at approximately 37C. One such pathway is the involuntary activation of skeletal muscle movement, a process referred to as shivering. This response can be elicited within minutes and progress to a more pronounced total body response indicative of severe shivering. Shivering requires a significant amount of energy and therefore generation of ATP to fuel muscle contractions. The fuel is initially generated from oxidation of glucose, followed by release of fatty acids due to lipolysis. As the severity and intensity of shivering progresses, breakdown of protein may further contribute to ATP production. In order to fulfill heat requirements following a prolonged and intense exposure to cold, there is a significant increase in energy expenditure, oxygen consumption, and carbon dioxide production. Despite the fact that cold exposure induces a significant increase in energy expenditure, shivering is uncomfortable and prolonged exposure to cold can ultimately lead to hypothermia, making this a less than favorable strategy for sustainable weight loss.

Non-Shivering Thermogenesis

A more palatable method of increasing energy expenditure is induction of non-shivering thermogenesis, which can be accomplished by activating brown adipose tissue (BAT). There are two main types of adipose tissue, white adipose tissue (WAT) which primarily serves as a storage depot for excess nutrients, while BAT, instead of storing energy, dissipates it through heat production. One of the hallmark characteristics of BAT is that it contains significantly more mitochondria than WAT which serves to generate production of heat through a protein located on the inner mitochondrial membrane called uncoupling protein-1 (UCP-1). Activation of UCP-1 uncouples oxygen consumption from ATP synthesis leading to dissipation of energy as heat. Further demonstration of the critical role for UCP-1 in maintaining body temperature are the observations that mice lacking UCP-1 have an inability following a cold challenge to maintain normal body temperature. WAT and BAT cells are further characterized by the fact that they are derived from different progenitors, with BAT cells coming from progenitors of skeletal muscle

and are positive for myogenic factor 5 (Myf5); whereas WAT cells are not. WAT and BAT are both innervated by sympathetic nerve efferent fibers, but BAT is more so than WAT and is characterized by increased vascularization to facilitate dissipation of generated heat.

BAT is abundant in small mammals or newborns who have a small body volume to body surface ratio and therefore have difficulty maintaining an adequate core body temperature through muscle shivering alone. One can speculate the large surface area to volume ratio in small mammals which renders them susceptible to substantial amounts of heat loss required development of a mechanism dedicated entirely to the production of heat and at the same time not interfere in other organ function such as skeletal muscle contraction. By contrast the small surface area to volume ratios in larger mammals results in less heat loss potentially explaining why BAT represents only a small portion of the total body mass in large mammals and adult humans. BAT in adult humans had long believed to be non-existent; however, with the use of positron emission tomography (PET) images as well as further analysis at the molecular level, it is now appreciated there is metabolically active BAT in humans. In addition, following cold exposure there is significant activation of BAT as demonstrated by the prevalence of BAT positive scans. Furthermore, short-term cold exposure appears to activate existing BAT depots; whereas more chronic exposure to cold can lead to expansion of the mass of BAT tissue. The location of BAT is distinct, and in humans, BAT has been identified in and around the aorta, common carotid artery, brachiocephaclic artery, paracardial mediastinal fat, epicardial coronary artery and veins, internal mammary artery, and intercostal artery and veins. Additionally, with use of magnetic resonance imaging (MRI), BAT has been detected in the intrascapular area both when measured at room temperature as well as following cold exposure.

Recently, research has focused on the fact there appears to be an additional type of fat cell which has now been identified and referred to as “beige” or “brite” adipose tissue. These cells differ from WAT and BAT, in that they are derived from progenitors different from the classical BAT myf-5 lineage, yet they have a significantly greater number of mitochondria and their function is more consistent with BAT than WAT. Beige cells can be identified by their distinct morphology in that they have a multilocular lipid droplet, higher mitochondrial content, higher levels of UCP-1, and a unique pattern of gene expression which differentiates these cells from both WAT and BAT. There is much speculation about the origins of these beige cells as well if these cells can revert back to WAT following warm exposure. Some studies suggest following cold exposure, these beige cells are derived from de novo differentiation from adipogenic precursor cells. What has been recently demonstrated is that following a second and more prolonged exposure to cold, these beige cells regain and maintain the morphological appearance of being multilocular which a signature gene expression profile consistent with beige adipocytes.

The mechanism by which one can illicit beiging of adipose tissues is through cold exposure and activation of the TRPM8 channel located in specific thermoreceptor neurons innervating the surface of the body, thereby signaling an increase in sympathetic nerve activity. Activation of the sympathetic nervous system facilitates the expansion of BAT and transformation of WAT to beige cells through stimulation of β-adrenergic receptors on adipocytes through cAMP-dependent protein kinase A and activation of the p38 MAPK pathway. Activation of these pathways leads to transcription and increased expression of UCP-1 and PGC-1α. The function of UCP-1 is to dissipate the proton gradient along the inner mitochondrial bilayer resulting in lipid oxidation being directed away from ATP synthesis and toward heat generation; whereas, PGC-1α enhances mitochondrial biogenesis.

The ability to acutely activate BAT following cold exposure can be accounted for by the densely innervated nature of adipose tissue. In order to account for expansion of thermogenic beige fat from more sparsely innervated WAT depots, studies have identified a pathway for local production of catecholamines orchestrated by type 2 immune cells and signals. In short, cold exposure activates eosinophils in adipose tissue leading to production of IL-4 and IL-13 and activation of catecholamine secreting alternatively activated macrophages. Under conditions of chronic cold exposure, increased arborization of nerve fibers can be demonstrated in the beiging fat depots mediated by adipocyte derived factors such as nerve growth factor, brain-derived neurotropic factor, and neuregulin 4. A similar program of acclimatization occurs with regards to vascularity of beiging fat. Cold exposure promotes angiogenesis by increasing the blood vessel density through increased expression of vascular endothelial growth factor (VEGF) thereby providing an avenue for heat dissipation.

The cellular energetics and fuel utilization of BAT and beige cells is similar. Following exposure to cold, BAT activation relies on fatty acids to directly activate UCP1 (Figure 3). In unstimulated BAT, fatty acids are predominately derived from triglyceride stored within BAT. Cold exposure increases lipoprotein lipase activity which facilitates uptake and utilization of plasma triglycerides. Cold exposure also upregulates norepinephrine which in turn, through a cAMP dependent mechanism, increases the amount of lipoprotein lipase gene expression. In fact, activation of BAT following exposure to cold can reduce hyperlipidemia and improve insulin sensitivity, further supporting the notion that activation of BAT and beige adipose tissues is an attractive method for improving insulin sensitivity and body adiposity without the requirement of caloric restriction. Specifically, adrenergic receptor activation causes upregulation and translocation of glucose transporters, GLUT1 and GLUT4, facilitating uptake of glucose into the cell thereby, improving insulin sensitivity and glycemia. Increased uptake and metabolism through glycolysis provides substrate for increased Kreb cycle activity and provides a mechanism for generation of ATP to offset the decline in production resulting from UCP-1-induced uncoupling in the mitochondria. Cell glucose can also undergo de novo lipogenesis to restore depletion of cell triglyceride content in activated BAT or beige cells.

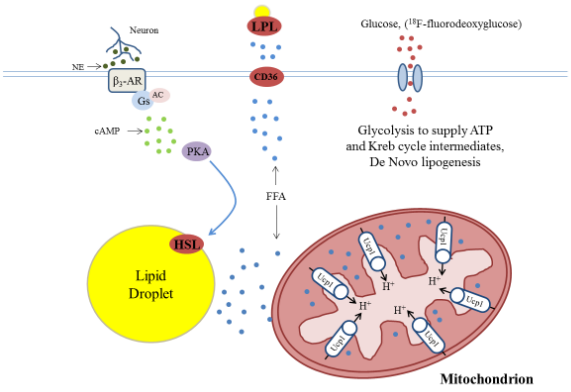

Figure 3: Activated brown/beige adipose tissue

Following cold exposure in humans, there is a significant increase in glucose uptake in BAT which appears to be associated with an increase in whole body energy expenditure, which in turn leads to weight reduction. Transformation of white/storage adipocytes into beige/burning adipocytes has received a great deal of interest as a method to increase total body energy expenditure and reduce body fat mass through enhanced lipolysis. Discussed below are circulating factors that have a role in transforming WAT into beige cells.

The natriuretic peptides are instrumental in maintaining extracellular fluid volume by regulating urinary sodium excretion; however germane to this discussion, the natriuretic peptides also have a role in promoting non-shivering thermogenesis by interacting with the sympathetic nervous system following a drop in ambient temperature. The natriuretic peptides also stimulate lipolysis through activation of cGMP-dependent protein kinase through NPR-A receptors on

adipocytes leading to activation of p38 MAPK resulting in an increased expression of UCP-1 and PGC-1α in adipose tissue. Furthermore, there is an increase in the circulating levels of ANP and BNP as well as the natriuretic receptors, specifically NPR-A, following cold exposure facilitating BAT activation and adaptive thermogenesis. Cold exposure also reduces the expression of the clearance receptor, NPR-C in BAT and WAT facilitating the ability of the natriuretic peptides to induce beiging. As mentioned previously, there is a requirement of the sympathetic nervous system to cause a similar shift in the ratio of the receptors.

An additional factor that induces beiging of adipose tissues and is induced by cold is fibroblast growth factor-21 (FGF21) which is most abundantly expressed in the liver with lesser amounts found in skeletal muscle and adipose tissues. In cold exposed humans, the diurnal levels of FGF21 are increased and correlate positively with an increase in energy expenditure. Levels of FGF21 correlate with BAT activity during acute cold exposure and parallel the increase in BAT activity following 10 days of cold acclimatization. In addition to acting centrally to increase sympathetic outflow, FGF21 acts in an autocrine/paracrine manner in BAT to activate thermogenesis and in WAT to induce the beiging process.

Does skeletal muscle 'beige' similar to what occurs in adipose tissues? Skeletal muscle is a site for non-shivering thermogenesis.

A great deal of interest has been directed to the role of skeletal muscle in non-shivering thermogenesis. Since skeletal muscle is the most abundant tissue in the adult human body, small changes in the rate of heat production would bring about substantial changes in whole body thermogenesis. As discussed previously the thermogenic property in BAT/beige adipose tissues is secondary to mitochondrial proton leak brought about by UCP1. Proton leak is also present in skeletal muscle and accounts for approximately 50% of resting muscle respiration and approximately 10% of total body resting metabolic rate, and this is generated through another uncoupling protein, UCP3. Given this large capacity, individual variability in the degree of proton leak may significantly influence the ability to lose weight. UCP3 is distinguished from UCP1 by its abundant and preferential expression in skeletal muscle.

The role of UCP3-induced mitochondrial uncoupling in skeletal muscle thermogenesis is however controversial. Muscle responds to cold exposure, and this is independent of shivering. In rodents UCP3 mRNA increases several fold in the first 24 hours of cold exposure but then decreases to 50% of control values after 6 days in the cold, a time period when non-shivering thermogenesis typically begins to increase. In humans, 60 hours of cold exposure actually reduced skeletal muscle UCP3 mRNA expression. In cold acclimated transgenic mice lacking UCP1 to minimize thermogenesis form BAT, there is no evidence of skeletal muscle mitochondrial uncoupling despite a 3-4-fold increase in UCP3 mitochondrial content and a switch towards increased capacity for lipid catabolism. While UCP3 may be able to uncouple oxidative phosphorylation and dissipate energy as heat, similar to what occurs in beige adipose tissues, this effect is likely secondary to its primary role which is to control mitochondrial reactive oxygen species production and regulate mitochondrial fatty acid oxidation.

Since altering mitochondrial leak as a mechanism to generate heat could adversely affect the availability of ATP necessary for skeletal muscle contraction, attention has turned toward changes in Ca^{2+} transport across the endoplasmic reticulum as a mechanism for skeletal muscle thermogenesis. Studies in fish and birds have identified sarcoplasmic reticulum Ca^{2+} cycling as a mechanism for heat generation that would not interfere in ATP synthesis (Figure 4). The importance of sarcolipin in skeletal muscle thermogenesis has been demonstrated in transgenic

animal models. Mice lacking sarcolipin when exposed to cold are not able to maintain core body temperature and develop hypothermia. The cold sensitive phenotype is rescued following muscle specific overexpression of sarcolipin in sarcolipin null mice. In addition, sarcolipin mediated skeletal muscle thermogenesis is recruited to a greater extent following cold exposure in mice lacking UCP1 or when BAT is surgically removed.

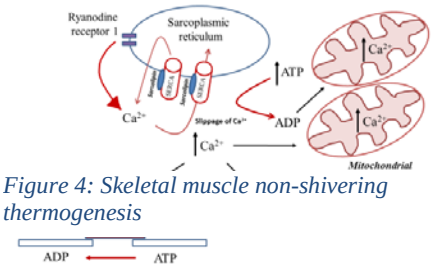

Figure 4: Skeletal muscle non-shivering thermogenesis

An intrinsic property of skeletal muscle is the ability to rapidly shift between fat and glucose as a preferred substrate to accommodate the needs of other organ systems. Sarcolipin increases the oxidative capacity of skeletal muscle and shifts substrate utilization toward a greater reliance on fat. Oxidative muscles are well suited to generate heat because of their ability to cope with greater energy demand while at the same time demonstrating less vulnerability to fatigue. This sarcolipin-induced shift in metabolism is accompanied by increased exercise endurance capacity when compared to control littermates.

Since BAT and skeletal muscle are derived from a common progenitor (Myf5 expressing progenitor), it is not surprising that they might share the common property of thermogenesis. As an example, similar to what was discussed above for the role of natriuretic peptides in inducing beiging of adipose tissues, natriuretic peptides also bind to receptors on skeletal muscle and directly enhance mitochondrial oxidative metabolism in association with increased expression of UCP3. While the method of heat production differs, both tissues are richly innervated, are capable of responding to circulating adrenergic factors, are mitochondrial rich, and both have a high capacity for lipid oxidation. While BAT is the primary source of heat production in small mammals, sarcolipin dependent non-shivering thermogenesis is dominant in animals where BAT is absent (birds and pigs) or where BAT content is reduced as in large mammals to include adult humans.

Can muscle thermogenesis be used as a method of sustainable weight loss?

Increasing skeletal muscle thermogenesis through upregulation of sarcolipin could be a potential strategy to promote weight loss. In response to a high fat diet, mice with skeletal muscle specific overexpression of sarcolipin consume more calories but gain less weight in comparison to wild type or sarcolipin null mice. When paired fed, the overexpressing mice lose weight compared to wild type while null mice gain weight. Overexpression of sarcolipin is associated with increased oxygen consumption and a lower respiratory exchange ratio consistent with a shift toward fatty acid oxidation. In muscle, both mitochondrial size and number are increased along with increased expression of peroxisome proliferator-activated δ (PPARδ) and PPAR γ coactivator α (PGC1α), key transcriptional regulators of mitochondrial biogenesis. These findings are consistent with an increase in sarcolipin-mediated futile cycling of the SERCA pump resulting in increased ATP hydrolysis. The increase in cytosolic Ca^{2+} facilitates entry into the mitochondria causing activation of mitochondrial oxidative metabolism and ATP synthesis. Elevated cytosolic Ca^{2+} initiates signaling pathways including calcineurin and Ca^{2+} calmodulin dependent protein kinases II which are important for programming the muscle into an oxidative phenotype through activation of PPARδ and PGC1α, putting this futile cycle as a novel and potential therapeutic option to increase energy expenditure in the face of caloric restriction thus creating an environment for sustainable weight loss.

Exercise and non-shivering thermogenesis

Exercise has been shown to induce markers of beige adipocytes in both visceral and subcutaneous fat depots but greater in the latter. It would seem paradoxical that exercise, with its attendant heat production, would be associated with induction of increased thermogenic capacity in adipose tissues. This response may have evolved as a way for skeletal muscle contractions associated with shivering to signal BAT expansion so as to increase the contribution of non-shivering thermogenesis to thermoregulation and lessen the dependence on shivering during long term cold exposure. Activation of sympathetic nerves, increased levels of natriuretic peptides, and/or increased circulating lactate levels are all factors associated with exercise which could bring about this response.

More recently, a number of myokines, or circulating factors released from muscle, have been identified that enable skeletal muscle to communicate with adipose tissue and initiate a thermogenic programming response. Specifically, the fibronectin type III domain containing 5 (FNDC5) gene encodes a skeletal muscle protein that is proteolytically cleaved into irisin. This protein is an exercise induced myokine that drives beiging of adipose tissues and UCP1 expression in the subcutaneous depot. Cold exposure induces irisin secretion in proportion to the intensity of shivering and of a magnitude similar to exercise stimulated secretion. Meteorin is another myokine that is induced after exercise and upon cold exposure and causes an increase in whole body energy expenditure associated with beiging of adipose tissues. Unlike irisin which can directly interact with receptors on adipocytes, meteorin causes the release of IL-4 and IL-13 from eosinophils embedded in white adipose tissue, which in turn activates local macrophages to release catecholamines. As discussed previously, local production of catecholamines from alternatively activated macrophages is important for the induction of thermogenic and β-oxidation genes in WAT and BAT upon exposure to cold.

Is there a sexual dimorphism of non-shivering thermogenesis?

There is a sexual dimorphism with respect to function, amount, and activity of beige/BAT adipose tissues. PET-CT imaging studies show women have greater quantities of BAT. A greater quantity of BAT along with higher expression of genes involved in mitochondrial function to include UCP-1 provide a mechanism for the higher metabolic rate per kilogram adipose tissue in women as compared to men. In fact, findings from a study where perirenal and subcutaneous adipose tissue was obtained from healthy live human kidney donors, brown-like adipocytes were present in women, but not men, when the average outside temperature is below 11C before harvesting. The sexual dimorphism therefore might suggest sex hormones may mediate these differences. One hypothesis being explored by many laboratories is might estrogens have an ability to increase resting metabolic rate and activate BAT thermogenesis, and can this be mediated either directly at the level of the adipocyte or indirectly through the central nervous system?

A number of studies suggest the local hormonal environment influences the thermogenic properties of the adipocyte. In cultured primary mouse BAT cells, estradiol suppresses transcription of the α-2 adrenergic receptor and decreases the α-2/β-3 adrenergic protein ratio. These changes facilitate adrenergic signaling leading to increased thermogenic activity and lipolysis since α-2 adrenergic stimulation gives rise to an antilipolytic effect by decreasing cAMP. Testosterone also appears to have a role, as there are data to suggest that it decreases lipolysis in these cells in association with up regulation in the mRNA levels and protein for the α-2 adrenergic receptor. Additionally, there are data to suggest female rats show morphologic differences in BAT

indicating a heightened thermogenic capacity as compared to males. These morphologic changes are evidenced by a greater size of mitochondria, greater cristae length, and higher mitochondria cristae density. In addition, levels of UCP1 per gram of adipose tissue and oxygen consumption are higher in female rats as compared to male rats.

In further support of a role for sex hormones in mediating these effects, castration of male mice increases body temperature and reduces body weight gain compared to sham operated mice, suggesting that testosterone may have an inhibitory role, and/or the lack of conversion of testosterone to estrogens following removal of the gonads may significantly impair this process. Specifically, it appears that these changes occur in association with increased mRNA expression of UCP1 in BAT in the castrated mice. By contrast removal of ovaries, ovariectomy, leads to increased food intake and body weight with evidence of increased caloric efficiency facilitating the weight gain. Estrogen deficiency has been demonstrated to decrease metabolic rate since estrogen deficient rats have to consume 16% less chow than ovarian intact sham-operated controls. In further support of a direct role for estrogens, UCP1 expression is reduced in BAT following ovariectomy when compared to intact or estrogen substituted rats suggesting estrogen deficiency decreases energy expenditure possibly through a reduction in BAT thermogenesis.

As discussed previously sympathetic nerve activity is a major regulator of BAT activity. Recent evidence suggests an adipose tissue depot specific sexual dimorphism may exist in recruitment of BAT adipocytes that is determined by an interplay between estrogens and sympathetic innervation. Administration of the β3-adrenergic agonist (CL316,243) induces the expression of markers of beige adipocytes in gonadal WAT (gWAT) in female but not male mice. As measured by tyrosine hydroxylase levels (a marker of norepinephrine turnover), the level of sympathetic nerve activity in this depot was greater in the females when compared to males. When ovarian failure was induced chemically in mice, the differences in sympathetic innervation and beiging between the sexes was no longer present.

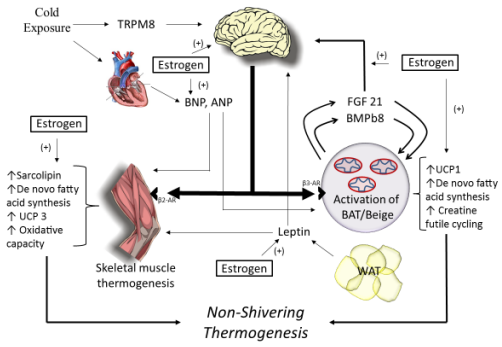

Figure 5: Sexual dimorphism in non-shivering thermogenesis

There are several potential mechanisms by which sex hormones many modulate these phenomena (Figure 5). Brain derived neurotropic factor (BDNF) is a factor previously found to stimulate beiging of adipose tissues, and expression levels are higher in the gonadal adipose tissue depot of female mice, but reduced following ovarian failure. Additionally, a direct effect of sex hormones, and specifically estrogens have been shown in primary cultured adipocytes, where 17β-estradiol increased BDNF levels. These data imply that there may be direct effects of estrogens and one that does not require the central nervous system.

Another factor linked to increased BAT thermogenesis is bone morphogenic protein 8b (BMP8b), and this also appears to be sexually dimorphic. Cold exposure increases BMP8b expression in BAT 35-fold higher in female mice when compared to males. Additionally, ovariectomy abolishes the expression of BMP8b in BAT but is restored following the administration of estrogen, further supporting a role for estrogens in modulating adipose tissue function.

Estrogens in addition to the direct effects on the adipose tissues, also have effects at the level of the brain/central nervous system to induce activation of BAT and beiging of adipose tissue.

This effect has been linked to the ability of estrogen to increase BDNF as well as exert a suppressive effect on AMPK in the hypothalamus. BMP8b also exerts a thermogenic effect centrally that is estrogen dependent and can be prevented when AMPK is persistently active.

There appears to also be a sexual dimorphic role for natriuretic peptides to promote nonshivering thermogenesis. Specifically, estrogens have a stimulatory role on ANP and BNP, and following menopause, males and females have similar levels of natriuretic peptides. In further support of an estrogenic role in regulating the natriuretic peptides, during the third trimester of pregnancy, basal metabolic rate progressively increases with an analogous increase in browning of adipose tissues mediated by ANP and BNP. Natriuretic peptides are also elevated in new born infants which is highly correlated with the increased levels of BAT which may result from placental production of ANP.

Sex hormones and their impact on skeletal muscle thermogenesis

There are not only sexually dimorphic effects at the level of adipose tissues, but there are also differences at the level of muscle. Specifically, skeletal muscle substrate utilization differs between men and women. Women have greater skeletal muscle uptake of plasma free fatty acids accompanied by higher intramyocellular lipid content and higher capacity for β-oxidation of long chain fatty acids, and during conditions of increased energy demand women rely on fatty acid oxidation more readily than men at the same relative submaximal exercise intensity. In attempts to determine the factors that diverge between men and women and drive these differences, men have been supplemented with 17β estradiaol for 8 days and following this, there is a reduction in the respiratory exchange ratio during exercise accompanied by increased β-oxidation capacity as measured in skeletal muscle biopsy specimens. Additionally, supporting a direct role of estrogens, administration of 17β-estradiol to ovariectomized ewes increases heat production in skeletal muscle without an associated change in blood flow suggesting the thermogenic response is a cellular event. To begin to unravel the mechanisms by which estrogens may do this, researchers have removed endogenous estrogens in ovariectomized animals and found that exogenous return of estrogen activates skeletal muscle AMPK which in turn inhibits acetyl-CoA carboxylase causing a decrease in malonyl-CoA levels and facilitating mitochondrial fatty acid use via increases in CPT activity.

Other factors differ between males and females such as leptin, which are higher in women than in men. Higher leptin levels are the result of greater fat mass in women and due to a direct effect of estrogen to increase leptin transcription. As previously discussed, leptin can directly activate BAT through central mechanisms as well as through effects at the level of the adipocyte; therefore, leptin may contribute to the sexually dimorphic response in BAT, beige potential, and skeletal muscle oxidation in women. It is not known whether the ability of estrogen to increase the oxidative capacity of skeletal muscle could give rise to a sexual dimorphism in the magnitude of skeletal muscle thermogenesis following cold exposure. What effect, if any, estrogens may have on sarcolipin expression is clearly of interest.

Conclusion:

There is currently an estimated rate of obesity prevalence which will top 60% of the US population with this affliction, thus popularizing shows such as the Biggest Loser. Sustainable weight loss is difficult to achieve, there are biological forces at play that enhance muscle and metabolic efficiency at a time in which weight loss is being attempted which thwart the

individual's ability to maintain and sustain any substantial weight loss. There are few pharmacological remedies for this, and to date, the most efficacious way to lose weight is through surgical options which have significant morbidity and mortality associated with them. It is time therefore, to begin to explore novel mechanisms by which to achieve sustainable increases in metabolic rate in the face of caloric restriction. This grand rounds discusses several mechanisms to achieve this to include ascent to altitude, exposure to cold, transformation of storage adipose tissues into metabolically active tissue through the process of 'beiging' of adipose tissue. Also discussed is a novel and potentially important concept, 'beiging' of muscle to increase futile energy loss while maintaining elevations in energy expenditure. There are sexual dimorphisms at play indicating one method of increasing and sustaining energy expenditure may not be equally achievable in both sexes. Now is the time to focus on methods to increase energy expenditure to facilitate sustainable weight loss.

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