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The role of beige fat in combating obesity

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Thesis

THE ROLE OF BEIGE FAT IN COMBATING OBESITY

by

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DEDICATION

I would like to dedicate this work to my late uncle, John Stibolt, who passed away in 2013 after a lifetime of making the people around him laugh.
THE ROLE OF BEIGE FAT IN COMBATING OBESITY

ROBERT DAVIS STIBOLT JR.

Boston University School of Medicine, 2015

ABSTRACT

As obesity and obesity-associated diseases become more prevalent in western societies, new methods to promote weight-loss and protect patients from the dangerous consequences of excess adipose tissue are needed. While both researchers and clinicians previously turned to chemical uncouplers for many decades to create a negative energy balance and thus promote weight-loss, these compounds proved to be extremely dangerous treatment options, even when taken in mild dosages. Substances like 2-4 dinitrophenol (DNP), were able to significantly induce weight loss, however many life-threatening conditions such as fatal hyperthermia are commonly attributed to these uncoupling agents.

Recently, with the discovery of natural brown/beige fat reservoirs in humans, many members of the medical community have become heavily invested in the targeting of more localized, less systemic uncoupling tissues. The action of UCP-1 in human thermogenic adipose introduces an opportunity to harness a natural, yet futile cycle, and hence boost a patient’s basal metabolism without ultimately compromising their long-term health. Many challenges remain before such a treatment is viable, including deciphering the biochemical pathways that induce brown fat thermogenesis. It appears
that several uncoupling signals may govern the genetic programs that lead to this thermogenic activity, and the “browning” of white adipose stores in humans.

Particularly in the last ten years, many studies have uncovered new components of the thermogenic program by ablating target genes in mice. While a direct pathway of thermogenic activation does exist when subjects are placed in a cold environment, a successful, high-adoption, anti-obesity treatment through a thermogenic regimen will likely involve a gene-therapy or protein-based biopharmaceutical intervention. It is conceivable that thermogenic manipulation could play a significant role in the battle against obesity and obesity-associated diseases, however a significant intellectual breakthrough in appetite suppression and/or appetite management (i.e. a successful intervention of the orexigenic and anorexigenic physiological pathways) could in theory supplant this approach.
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LIST OF COMMON ABBREVIATIONS

ADA ................................................................. American Diabetes Association
Aдрb3 ................................................................. B3-Adrenergic Receptor
BAT ................................................................. Brown Adipose Tissue
BMI ................................................................. Body Mass Index
BRFSS ............................................................. Behavioral Risk Factor Surveillance Systems
CDC ......................................................... Centers for Disease Control and Prevention
DNP ................................................................. Dinitrophenol
EE ................................................................. Energy Expenditure
ETC ................................................................. Electron Transport Chain
FDG ................................................................. Fluorodeoxyglucose
FDG ................................................................. Fluorodeoxyglucose Positron Emission Tomography
iWAT ................................................................. Inguinal White Adipose Tissue
MRTFA ............................................................ Myocardin Related Transcription Factor A
NRFs ................................................................. Nuclear Respiratory Factors
PET ................................................................. Positron Emission Tomography
PPARα .............................................................. Peroxisome Proliferator Activated Receptor-α
SC ................................................................. Sub-Cutaneous
UCP1 ................................................................. Uncoupling Protein 1
WAT ................................................................. White Adipose Tissue
INTRODUCTION

Background

The increasing prevalence of obesity and Type-2 Diabetes in the United States is fueling research into alternative methods for weight loss. While general appetite suppression continues to be an area of high scientific interest, recently there has been a growing initiative to address these diseases by safely boosting the body’s available metabolism. Thermogenic fat, particularly “brown” or “beige” fat, may be an emerging avenue to treat patients who either suffer from obesity directly, or from diseases that are heavily associated with an unhealthy level of body fat (Virtanen et al., 2009). Although it was known that human infants possessed thermogenic fat—a likely physiological defense against their predisposition to lose heat to their surroundings—it was not until fairly recently that scientists found evidence of brown/beige fat in adults (Rousseau et al., 2006).

In 2009, scientists using Positron Emission Technology (PET) scanning discovered deposits of thermogenic fat in the supraclavicular and spinal regions of breast cancer patients. In total, within a portfolio of over one hundred breast cancer patients, nearly half of the PET and computerized tomography (CT) scans revealed significant Fluoro-D-glucose uptake in the supraclavicular areas (Virtanen et al., 2009; Rousseau et al., 2006). Further biochemical and histological examinations of these stores revealed that this tissue was adipose in nature, expressing the typical markers, such as the mitochondrial associated Uncoupling Protein 1 (UPC-1), found in the brown fat of rodent models. While Cypess et al. have suggested that maximally activated brown fat depots
could burn up to 400 kcal per day, much larger potential benefits may lie in the ability of these tissues to actively consume free glucose and burn circulating triglycerides (Cypess et al., 2014). In theory, if thermogenic fat could be harnessed effectively in overweight patients, this would provide an excellent therapeutic supplement alongside an appropriate combination of careful diet and exercise.

**Figure 1** reveals two regions within the body that appear to house brown adipose tissue, which become metabolically activated when a patient is exposed to cooler temperatures.

**Developmental Origins**

Although mammals rely on stored chemical energy to execute both intracellular and extracellular functions, heat is also a critical product of energy release that is harnessed to maintain an appropriate internal temperature. When chemical energy is not used for work functions, it may be called upon to elicit a higher body temperature through a series of heat-generating pathways (Kajimura and Sidossis, 2015). Over the course of mammalian evolution, thermoregulation consequently allowed early mammals to simultaneously seek food and avoid predation in areas that were previously inaccessible by ancestral counterparts (Grady et al., 2014). Although the best characterized pathways in mammals involves UCP-1, it is believed that there may be many more pathways that help a mammalian organism evade hypothermia, when environmental conditionals are appropriate for such a response. The idea of multiple pathways is supported by an early demonstration that polarity-regulating kinase partitioning-defective 1b (Par-1b) and microtubule affinity-regulating kinase 2 (MARK2)
Figure 1: Human Brown Adipose Tissue Deposits

Imaging provided by Masayuki Saito, PhD (Contact: saito@tenshi.ac.jp) with expressed written permission. Brown/Beige fat is detected by fluorodeoxyglucose (FDG)-positron emission tomography (PET) when a human subject is placed in a cold chamber (19°C) for 120 minutes. It predominates in a supraclavicular and paraspinal region of the body, and is not readily seen when placed at warmer temperatures, such as 27°C for the same time interval (Saito, 2013).
omissions in knockout mice significantly increased energy expenditure without altering UCP-1 production (Huroy et al., 2007).

While brown adipose tissue (BAT) animal models primarily achieve energy/heat dissipation through this UCP-1 route, there is an intriguing subset of white adipose tissue (WAT) that also expresses the UCP-1 protein under a defined set of conditions. This will be discussed further in greater detail. Nevertheless, when scientists assessed the initial BAT found in perithyroid cervical adipose biopsies, there was an immediate interest into the developmental origins of the BAT cells. In 2009, Zingaretti et al. concluded that because newborns’ brown fat developed before the white fat, there were essentially two conceivable ways in which this transition could be explained: either the BAT deposits are progressively infiltrated by WAT, or the previous BAT somehow transdifferentiates into white adipose. Whether or not this differentiation was permanent, or reversible, was also a point of great interest and debate. Despite many attempts to ascertain where these cells came from, and why they subsequently disappear, at present there is an insufficient amount of data to definitively suggest the true origins. To date, all indications are that the progenitor cells for WAT and the beige cells found in humans, share a common vascular origin (McDonald et al., 2015).

**Reclassification in Humans**

In 2012, Dr. Bruce Spiegelman of the Dana-Farber Cancer Institute published a paper in *Cell*, in which he and his colleagues introduced the idea that the brown fat surrounding the neck and spinal regions in adult humans should be coined a “beige” adipocyte. Unlike traditional thermogenic fat from animal models, Spiegelman et al.
noticed that these beige cells, previously identified by Cannon and Nedergaard, lacked many of the gene products of brown adipocytes, and had varying expression of UCP1. Moreover, questions were raised as to the lineage of these beige cells, which did not appear to share a myf-5 lineage via the “PR domain containing 16” gene’s (PRDM16) transcriptional regulator (Wu et al., 2013; Cohen and Spiegelman, 2015). This discovery connected previous observations made by other biochemists on what they called “brite” or “beige” fat tissues, which could be invoked within WAT cell populations when treated with rosiglitazone, a known peroxisome proliferator-activated receptor gamma (PPARy) agonist.

Furthermore, while these beige cells have significant UCP1 expression and a high concentration of mitochondria, the basal UCP1 activity was significantly lower than traditional BAT. This suggested that beige fat was in fact a hybrid form of tissue, which was confirmed when Wu and Boström found that human tissue extracted from what was previously viewed as “brown” fat had a distinct expression profile, differing from both BAT and WAT populations (Wu et al., 2012). Perhaps the most important discovery in Spiegelman’s work was not simply the distinct labeling of the fat, but rather the observation that forskolin could raise intracellular cyclic AMP (cAMP), and achieve maximal UCP1 expression to rival levels found in BAT. In terms of the therapeutic potential of beige fat in humans, the idea of activating thermogenic stores using an underlying pathway remains paramount.
Relevance

According to the American Diabetes Association’s (ADA) “Economic Costs of Diabetes in the U.S. in 2012,” the total cost of diabetes in the United States is well over $200 billion annually (Barker et al., 2012). Consequently, many large pharmaceutical companies are heavily invested in finding a safe and effective drug to promote weight loss in overweight patients. Despite some clinical efficacy with regimens that target the central nervous system to suppress appetite, many of these therapies have undesirable side effects, and are also plagued by issues of desensitization and rebound withdrawals (Branis and Wittlin, 2015). In fact, drugs that treat insulin resistance and cardiovascular disease—two conditions that are strongly linked to excess adiposity—may disrupt a patient’s physiological homeostasis, leading to hospitalizations and even death (Gu and Xu, 2013).

On the other hand, thermogenic fat represents a chance to tackle obesity using a more localized, peripheral target. This essentially decreases many of the unwanted interactions with other organs and tissues, while simultaneously providing a treatment that would conceivably act more in the manner of a light-switch, rather than an oven. This paper aims to discuss known stimulators of beige fat, the pathways and transcriptional cascades that underlie such stimulation, and ultimately predict whether or not thermogenic fat is a viable solution to treat obesity and type 2 Diabetes.
THERMOGENIC STIMULI

Overview

In order for mammalian organ systems to functional optimally, the body as a whole must be maintained within an acceptable temperature range. When the external environment is not conducive to such a homeostatic window, an organism may undergo a series of behavioral and/or physiological changes in order to re-align the core temperature (Abreu-Vieira, 2015). In the event that the core temperature falls below the desired set points, one of the most observable physiological responses involves an involuntary shiver. These shivers involve a series of random muscle twitches, increasing the metabolic activity of the musculoskeletal system, and thereby forming excess heat to compensate for the depressed core temperature.

However, though shivering and other behavioral reflexes are the most obvious responses to a cold scenario, in reality there are other internal responses at play. In short, adipose tissue plays an important role in thermoregulation, not only serving as an added layer of insulation between the internal compartment and the environment, but also executing a series of biochemical programs to generate body heat. Moreover, while these steps are only minimally understood, in recent years there has been an increasing drive to uncover how subcutaneous fat is stimulated, and how this stimulation is achieved through cell-signaling pathways (Wu et al., 2013). The following section addresses the known scenarios that cause thermogenic fat to elicit a response in vivo.
Cold Exposure

While the sympathetic adrenergic pathway and Beta 3 receptor are known to play an integral role in sensing a thermogenic stimulus, in 2013, Ye et al. discovered that UCP1 expression could be induced in mouse models lacking all sets of Beta-Adrenergic receptors (β-AR) (Ye et al., 2013). This finding is intriguing, as it suggests that there is a B-AR-independent pathway for achieving the thermogenic program. In other words, in this case the cold temperatures (20 hours of exposure at 10 degrees Celsius) were directly stimulating the fat cells to commit to thermogenic gene expression.

This form of activation is in fact limited to subcutaneous (S.C.) fat, and is not found to the same extent in visceral depots (Ye et al., 2013). Direct subcutaneous stimulation by a cold environment seems like a plausible physiological activity, seeing as it is conveniently positioned to experience rapid changes in environmental temperature. This in essence introduces an innate ability of S.C. tissue to regulate its activity. In fact, the temperature of subcutaneous fat varies greatly by cold exposure, deviating as much as 15-20 degrees in humans (B-52). Deeper, viscerally located fat is not able to gauge temperature in such a manner, and hence this may explain why Ye et al. did not observe thermogenic activity in other adipose sources.

An autonomous activation of thermogenic programs, without the need for sympathetic innervation, has broad consequences for treating diseases that stem from obesity. It has been well-documented that humans with prolonged exposure to cold conditions, whether living win cold climates, winter conditions, or laboring in cold outdoor conditions, have elevated levels of beige fat in their adipose tissue (Ye et al.,
2013; Cypess et al., 2009; Saito et al., 2009). In addition, the brown fat that is housed by patients who fit these conditions is more readily activated, initiating a thermogenic repertoire at a much faster rate when compared to comparable tissues in patients of warm environments (Ye et al., 2013, Saito et al., 2009). This direct activation pathway raises questions as to whether placing an overweight patient in a sufficiently cold environmental is a viable route to promote weight loss through thermogenic activity. Though placing a patient at an undesirable temperature for a prolonged period of time may seem impractical, the reality remains that this manner of activation may be far less harmful than alternative routes, which may impact other organ systems and incur collateral damage to the body’s natural physiology.

**PPARα Agonists**

It was previously theorized that the glucose and lipid metabolic nuclear receptor “Peroxisome Proliferator Activated Receptor-α” (PPARα) was responsible for repressing a portfolio of muscle-associated genes, leading to the formation of brown adipose tissues in-vivo (Tong et al., 2005). However, in a 2010 Biochemical and Biophysical Research Communications article, Walden et al. demonstrated that this observation was inaccurate, and that PPARα likely influences brown adipocyte formation through a set of regulatory genes: Zic1, Lhx8, and Prdm16 (Petrovic et al., 2010). Moreover, there was debate over whether these genes are able to manipulate the fate of the adipomyocte precursor cells, by down-regulating myogenic factors that aid the commitment of these precursor cells to muscle tissue, in turn opening a window for brown adipose development (Petrovic et al., 2008). Although intuitively, it would seem that such a down-regulation would involve an
inhibitory repression of the muscle-associated differentiation program, Walden et al. suggest that the action of PRDM16 takes place after the developmental bifurcation (Petrovic et al., 2010).

When the PPARα agonist Wy-14,643 is administered in wild-type mice, muscle-associated mRNA (e.g. tropomyosin-β, myosin regulatory light chains) were reduced by 50% when compared to no-treatment controls. In addition, when this same agonist is administered to PPARα-ablated mice, no noticeable change in mRNA was detected. When this observation is combined with the fact that PPARα-ablated mice show lower levels of norepinephrine-induced UCP-1 expression, it is evident that this route is clearly involved in the development of thermogenic fat tissue (Petrovic et al., 2008). However, the exact means remains highly uncharacterized and controversial, and therefore this stimulus response is an area that is in need of further ablation-based studies.

**Beta-3 Adrenergic Agonists**

The β3-Adrenergic Receptor (Aдрb3) is a G protein-coupled receptor, which when intentionally stimulated for a prolonged period of time, has been observed to possess both anti-obesity and anti-diabetes potential in mouse models (Granneman et al., 2005). Aдрb3 is believed to exhibit its effects by increasing the metabolic activity of white fat, specifically though an oxidation of fatty acids (Wojtczak & Schonfeld, 1993; Zingaretti et al., 2009). However, although Granneman previously demonstrated that this up-regulation of basal metabolism was independent of the UCP-1 pathway, Aдрb3 has been shown to trigger the mitochondriogenesis of multilocular adipocytes, and therefore this
may suggest that uncoupling of oxidative phosphorylation is involved (Wojtczak & Schonfeld, 1993).

Other researches such as Carmen Guerra have similarly suggested that A德拉3 stimulation may be at least partially responsible for the induction of brown fat within white adipose deposits (Guerra et al., 1998). However, there is still great debate as to whether such an induction is produced by compartmentalized stem-cell proliferation, or alternatively by specific white adipocytes that may acquire brown-like characteristics. In summary, A德拉3 is clearly an ongoing target of interest in terms of its potential therapeutic role in fighting obesity and type II diabetes. Unsurprisingly, recent genetic studies in Europe on Hungarian school-aged children have revealed a link between A德拉3 allelic polymorphisms and corresponding polymorphisms amidst uncoupling proteins (Csernus et al., 2015). These overlapping polymorphisms were correlated with higher Body Mass Indexes (BMI) and insulin resistance, suggesting that many of the abovementioned stimulus pathways are intricately interconnected.

**FGF21**

FGF21 is a major regulator of several physiological processes, chiefly including glucose homeostasis, as well as the body’s biochemical response to insulin. While most FGF21 has traditionally been attributed to the liver, many researchers including Hondares, Iglesias, and Villaroya have identified that thermogenic adipose tissue, upon activation, becomes a systemic source of FGF21, through a cAMP-governed pathway. Norepinehprine, through β-adrenergic mechanisms, upregulates both transcription and
release from brown adipocytes, creating a possible endocrine role for BAT, especially active in cold-induced interscapular brown adipocytes. This is particularly captivating, as WAT has previously been known as the primary adipose tissue with a multitude of endocrine functions. In terms of FGF21’s relevance to the battle against obesity and obesity-associated diseases, some researchers have hypothesized that the release of FGF21 during the thermogenic repertoire allows the brain (as well as peripheral tissues) to effectively mobilize glucose to meet the increased energy demands of the body (Rousseau et al., 2006; Hondares et al., 2011). This aspect of the thermogenic activation of adipose tissue is therefore of particular interest, as the mobilization could theoretically contribute to a negative energy balance.

**Irisin**

Irisin is induced during chronic exercise in rodent populations, and also appears to brown white fat deposits when introduced to mouse models through methods of viral infection (Erickson, 2013). In fact, several 2013 publications by Park and Choi revealed a correlation between diabetic patients and Irisin deficiencies, and therefore the involvement of Irisin as a thermogenic stimulus is intriguing from a therapeutic vantage point. The exact means by which Irisin achieves a rapid response (<20 minutes) is currently unknown, although such a rapid response suggests that there is a membrane-bound receptor in many cell-types (Erickson, 2013; Gannon et al., 2015).

However, until this receptor is located, it is unlikely that Irisin investigations will be fruitful in terms of their therapeutic potential for obesity and Type-II Diabetes. To make matters more convoluted, in a 2013 article within *Adipocyte*, Harold Erickson
explored the popular alternative that Irisin is actually an exercise hormone that is cleaved into circulation (Erickson, 2013). Until these diametrically opposed vantage points are resolved, other thermogenic targets within humans must be investigated. In conclusion, a breakthrough in Irisin receptor/hormone theory would be significant for the further exploration of thermogenic fat activation. Figure 2 (Below) shows the general structure of the irisin dimer, a proteolytic product of the FNDC5 mRNA product.

**Cardiac Natriuretic Peptides**

A 2012 exploration of the role of Cardiac natriuretic peptides in the brown fat thermogenic sequence by Bordicchia et al. revealed an intriguing role of these peptides in beige-trait acquisitions. In summary, it appears that both atrial and ventricular NP’s induce UCP-1 expression, recruit the formation of additional mitochondria, and increase both total respiratory activities and uncoupling actions (Zingaretti et al., 2009; Bordicchia et al., 2012). These observations are strengthened by the observation that low concentrations of natriuretic peptides have profound consequences on many thermogenic fat and mitochondrial markers. Furthermore, when the blood of cold-exposed mice is sampled, circulating NPs are abundant, with two notable changes in NP receptor expression. First, NP signaling receptor is increased, allowing cells to recognize the circulating natriuretic peptides with increasing frequency. Secondly, expression of the NP clearance receptor is diminished, allowing for these peptides to have prolonged action on the relevant BAT and WAT populations.
Figure 2: Irisin Dimer Structure, courtesy of Nevit Dilmen (Nevit, 2015). Irisin was recently believed to be a cleaved product of the FNDC5 protein, forming an extensive inter-subunit beta-sheet. However, recent evidence suggests that perhaps Irisin does not play a physiological role in the circulation. The “FNIII-like fold” previously suggested that the dimer is adept for ligand/receptor activation. However, the receptor structure remains a controversial topic of conversation. This figure illustrates some of the biochemical complexities behind thermogenic substrate-receptor mechanisms.
Remarkably, adding ventricular BNP into mice greatly increases thermogenic gene expression, energy expenditure, and respiratory activity (Bordicchia et al., 2012). While it is largely unknown whether natriuretic peptides are a viable therapeutic avenue in human patients, (considering the systemic repercussions of exploiting these peptides, which have significant cardiovascular and renal effects) it is a both novel and fascinating concept to view the heart as a significant regulator of the body’s adiposity.

THERMOGENIC PATHWAYS

Preview

To briefly recapitulate, interscapular BAT appears to originate from the dermomyotome, while beige fat has a smooth muscle origin, suggesting a close association with the vasculature (McDonald et al, 2015; Seale et al., 2008). When considering the pathways involved in producing beige/brite fat-like deposits, it is pivotal to consider the changes in cell morphology that ensue when MSCs commit to such a lineage. In turn, excess adiposity (and thus macroscopic outcome of obesity), is often characterized by inflammation, and the creation of fat cells that can be potentially harmful not only from physical tissue contact, but also from endocrine secretions that may compromise the delicate internal homeostatic environment.

TGF-β vs. BMP7

In 2015, McDonald et al. investigated the role of Myocardin-Related Transcription Factor A (MRTFA) in the manipulation of progenitors into beige adipocytes, which intrinsically possess high amounts of uncoupling activity into the cell’s
Figure 3: Natriuretic Peptide Clearance Receptor Deficiency, adapted from “Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes, Journal of Clinical Investigation, Mar 2012, Bordicchia et al. These data suggests that removing the NP clearance receptor allows for a prolonged substrate-receptor interaction, presumably increasing lipolysis and WAT/BAT depots. However, it should be noted that UCP-1 and PGC-1α expression did not decrease; in fact, despite the drastic reduction in both stores, thermogenic expression actually increased, along with respiratory activity and total energy expenditure. The figure additionally illustrates the stark differences between wild-type (NPR-C+) mice and ablated (NPR-C-) mice, which appear to have significant protection from obesity.
metabolic state (McDonald et al, 2015). Their publication in Cell elaborates upon an interesting concept: the notion that cell morphology is altered by a series of underlying pathways. The publication describes a biochemical “tug-of-war” of sorts between two factors: TGFβ and Bone Morphogenetic Proteins (BMPs).

**TGF-β**

The Transforming Growth Factor Beta (TGF-β) superfamily of secreted proteins play important roles not only in immunological responses such as inflammation, but also in the overall coordination of the microvasculature (Jin et al., 2014). As discussed previously, the vasculature origin of beige adipocytes has broad implications in terms of potentially intervening in obese patients using biochemical or pharmacological resources. TGF-β is involved in the negative regulation of WAT browning. Morphologically, these TGF-β-influenced cells take on an elongated and filamentous (high actin) cytoskeleton, acting through a co-effector (Smad3) to inhibit differentiation. The Smad3 complex acts through a C/EBP-β (See subtopic “Other Transcriptional Areas of Interest” for additional information) intermediate, binding and repressing the relevant target gene sequences (McDonald et al, 2015; Choy et al., 2003). As discussed in the McDonald et al. publication, it has been observed that Smad3 ablated mice develop substantial depots of “brown-like” adipocytes in white fat stores, indicating that the TGF-β superfamily is involved in this departure from a thermogenic subset (McDonald et al, 2015).
BMP7

In sharp contrast with the actions of TGF-β on adipocyte precursors, bone morphogenetic proteins play a role in promoting adipose formation, and furthermore seem to drive MSCs to a brown, thermogenic lineage by promoting Prdm16 and PGC1-α expression. Presumably, this action is mediated through the Rho-associated protein kinase (ROCK), which BMP7 represses, in turn increasing the ratio of G-actin to F-actin. When this ratio increases, SRF genes are down regulated, blocking an important factor called MRTF-A, which will be discussed in greater detail below (Farmer, 2014). Unlike TGF-β’s effects on MSCs, ablation of the BMP7 activity dampens brown formation, providing a seemingly equal yet opposite measure to control the thermogenic potential of body fat. Additionally, it is particularly fascinating how strong the repression of ROCK by BMP7 is; from a biochemical perspective, McDonald et al. revealed that this inhibition is as effective as a specific inhibitor (Y27632) on ROCK (McDonald et al., 2015).

Figure 4 (below) illustrates histological differences between large unilocular droplets, and multilocular fat cells, which have likely acquired excessive thermogenic dispositions through mutations in the aforementioned mechanisms.

MRTF-A

There are several takeaways when examining the relationship between BMP and TGF-β directed manipulation of the MRTF-A pathway. The intense interest in the inner-workings of how different biochemical factors exert an effect through the intracellular pathways (e.g. p38MAPK signaling, SRF activity) is spurned by intriguing physiological
Figure 4: Histological Micrograph of a Benign Brown-Fat Tumor (Photo courtesy of Nephron 2009). In this hibernoma, the presence of multivacuolated, eosinophilic cells (examples indicated by black arrows) are the result of uncontrolled stimulation of fat precursor cells toward a BAT route. Though this form of tumor (soft-tissue in nature) is uncommon, its restriction to the subcutaneous fat is consistent with human BAT distributions, a defining characteristic of this tumor.
findings, such as those described in McDonald et al.’s MRTF-A deficient mice studies. In total, these mice had lower fasting levels of glucose, lower leptin levels, and a considerable protection from High-Fat Diet (HFD)-induced obesity. In addition to these hallmarks, insulin resistance, eWat inflammation, and the chronic accumulation of hepatic liquids were all considerably lower. Collectively, these outcomes are desirable in current patient populations who suffer from obesity and obesity-associated diseases, and therefore it is pivotal to further investigate any treatments that may translate into human models.

**Review**

To conclude, the thermogenic stimuli, as well as recent theories on how these stimuli lead to biological changes in-vivo were previously discussed. The next sections will examine the underlying mitochondrial action of uncoupling, and then the transcriptional cascade. Emphasis will be placed on the events that may be responsible for bridging the separation between the nuclear and mitochondrial genome.

**MITOCHONDRIAL FUNCTION**

**Overview**

Within the mitochondria of traditional energy-producing cells, sugars and fats are oxidized, freeing high-energy electrons that flow along an Electron Transport Chain (ETC). Protons are then pumped across the inner mitochondrial membrane, producing a gradient that is coupled to ATP synthesis on ETC complex V (Chan et al., 2006). In
UCP1+ cells however, this gradient is disturbed by a leak of protons back across the inner mitochondrial membrane by the protein. In effect, this uncoupling phenomenon is extremely energetically expensive, driving a proton cycle that is not used to create or transfer chemical energy, and hence cannot perform a cell’s required work functions. As a result, heat is constitutively formed by fuel oxidation, providing a means to deplete both glucose and fatty acids without the simultaneous formation of ATP.

Without adequate levels of ATP formation, life cannot be sustained in biological systems, and therefore uncoupling ATP production from oxidative phosphorylation can have lethal effects in vivo. Over 80 years ago, the renowned uncoupler 2-4 Dinitrophenol (DNP) was used to promote weight loss through this pathway. Insufficient ATP levels also mandate that excess sugar must be metabolized for energy needs, and therefore uncoupling ATP production from ATP synthesis was a means to boost carbohydrate catabolism. However, the safety margin for using DNP is extremely small, as the lethal dose and therapeutic dose occur within a small concentration range (Bethesda, 2015). Since the uncoupling actions of UCP1 are more localized, and do not effect essential non-adipose tissues, thermogenic uncoupling via this route is far more favorable in terms of its therapeutic potential.

**UCPs**

Uncoupling proteins are small, mitochondrial-based, membrane-spanning proteins that are found in a variety of tissues including adipose deposits and skeletal muscle (Wu et al., 1999). Adaptive thermogenesis not only introduces the action of mitochondrial uncoupling, but also orders a drastic increase in the number of active mitochondria, as
well as other adaptations to the electron transport chain (Cunningham et al., 1987). Collectively, these alterations increase oxidative activity and heat production, without compromising sensitive ATP/ADP ratios, which govern many essential signaling mechanisms such as cytoplasmic calcium levels (Nilsson et al., 1996).

Wu et al. confirmed the up-regulation in overall fuel oxidation through the use of carbonylcyanide-4-trifloromethoxyphenylhydrazone FCCP, an agent that can block all uncoupling proteins, permitting the direct measurement of electron transport only. When PGC-1 positive cells (see Figure 5 on page 22) were treated with FCCP, oxygen consumption was nearly two-fold compared to a non-PGC-1 control. Furthermore, when phosphorylation-linked respiration was blocked using Oligomycin, PGC-1+ cell lines consumed over three-times more oxygen; this suggests that PGC-1 may be a two-dimensional regulator of adaptive thermogenesis through the combination of mitochondrial uncoupling with increased aerobic activity (Wu et al., 1999).

**Transcriptional Control**

Since the aforementioned PGC-1 is restricted to the nucleus, there is a very intriguing relationship between the cell nucleus and the separate, mitochondrial genome, which undergoes a compartmentalized transcription and replication process that is outside the nuclear activity (Wai et al., 2008). Two families of transcriptional proteins, including nuclear respiratory factors (NRFs) and mitochondrial transcription factor A (mtTFA), may be responsible for calibrating mitochondrial expression. Likewise, there is evidence to suggest that this genetic interplay may at least partially involve PGC-1 as a key
Figure 5: PGC-1 Effect on Total Respiration Capacity, adapted from “Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator PGC-1, Cell, July 1999, Wu et al. These data suggest that uncoupling involves a recruitment of additional mitochondria to maximize fuel oxidation rates. The differences between PGC-1+ and PGC-1(-) fuel oxidation, additionally suggests that PGC-1 is an integral regulator of brown adipose metabolism.
modulator. This transcriptional activity will be discussed in greater detail, in the “transcriptional cascade” section below.

TRANSCRIPTIONAL CASCADE

Overview

The transcription factor and coregulatory protein Prdm16 likely plays a primary role in the browning of white adipose cells, first by manipulating the expression of thermogenic gene sequences such as the PGC1-α route, and secondly by influencing a mitochondrial cascade. These mechanisms, along with other recent findings, will be discussed below.

PRDM16

At present, scientists are uncertain as to the exact relationship between transcriptional events between brown and beige fat cells. While Prdm16 is clearly an integral transcription factor that is responsible for initiating the thermogenic gene sequence, it is not known whether Prdm16 plays a role in BAT development in vivo. However, several authors have demonstrated that ablating the Prdm16 sequence in mice results in an acute decline in interscapular BAT activity. In 2014, Harms et al. showed that the BAT loss in Prdm16 deficient mice was amplified when a homolog Prdm3 region was also deleted (Harms et al., 2014). Therefore, although it appears that both brown and beige fat depend on these factors to develop thermogenic repertoires, Prdm3 may serve an auxiliary role.

While a postnatal role of Prdm16 has only been minimally investigated, in mice, it appears to influence the muscle vs. brown fat commitment of precursor cells, prior to the two-week point of gestational age (Seale et al., 2008; Cohen et al., 2014). Conversely,
although the beige/brite cells from WAT do not share this Myf5+ lineage, Prdm16 still appears to influence the adipose content of the body, by influencing the metabolic activities of Subcutaneous versus visceral fat.

**PRDM16 Deletion**

When Cohen et al. ablated the Prdm16 coregulatory protein, knockout mice markedly lost the expression of a panel of thermogenic and mitochondrial ETC genes, including PGC1-alpha, UCP-1, CoxIII, Cox5b, and Cox8b. In addition, although these effects were seen to some extent in visceral fat depots, the subcutaneous adipose tissues were incredibly affected by this knockout scenario. In fact, when either treated with cold exposure or the Beta-3 adrenergic agonist “CL 316,243,” control (non-knockout) groups of mice exhibited a 70-fold (cold exposure) and 400 fold (β-3 Agonist) induction of UCP-1 (Cohen et al., 2014).

Interestingly, a transcription factor called Ebf2 appears to govern transcriptional differences between beige and brown adipocytes, attracting specific promoters including PPAR sequences upstream of the Prdm16 sequence (Harms et al., 2014). While the interconnectivity of these factors is not well characterized, the relevance of Prdm16 and Prdm3 and their roles in expressing PGC-1α and other thermogenic genes—including mitochondrial genes—is now widely recognized within the scientific community.

**Other Transcriptional Areas of Interest**

It is evident that Prdm16 protein product is able to bind CCAAT enhancer binding protein B, as well as two additional corepressor proteins which either down regulate the
expression of white adipose factors, as well as muscle genes (Cohen et al., 2014). This aspect of Prdm16 activity is intriguing, as it provides a logical route whereby thermogenic fat is not only promoted, but other cellular commitments are discouraged. Additionally, expression of TLE3 can repress thermogenic activity in both brown and beige cells, providing another avenue of cell-specific uncoupling altogether. In 2011, Villanueva et al. suggested that this factor acts as a “dual-function switch,” with both active and repressive complexes that are intricately influencing the fat programs, presumably through the PPARγ pathway (Villanueva et al., 2011).

Mitochondrial Influences

Puigserver et al. introduced an interesting finding in 1998, in suggesting that the PGC-1 product was confined to the cell’s nucleus (Wu et al., 1999). Their strong evidence consequently extended a pertinent question as to how the protein could manipulate the mitochondrial genome without exiting the cell membrane. Several previous authors previously identified a set of transcriptional factors with mitochondrial involvement, known as nuclear respiratory factors or NRF’s. In addition, Cohen recently found evidence that both NRF’s and mitochondrial transcription factor A (mtTFA) may be targets of PFC-1 (Cohen & Spiegelman, 2015). MtTFA is initially encoded in the nucleus, but translocates to the cellular mitochondria and asserts an influence over the D-loops of this mitochondrial DNA. Without mtTFA, mouse models experience a lethal reduction of mitochondrial oxidative phosphorylation and respiratory action, and therefore it is plausible that PGC-1 is able to affect extranuclear mitochondrial activity.
through this median (Cohen & Spiegelman, 2015; Wu et al., 1999). However, as more research is dedicated into the regulatory mechanisms and transcriptional cascade of thermogenic fat, it is increasingly apparent that a cassette of proteins may be at play.

**Nuclear Respiratory Factors**

In some recent literature, Cohen & Spiegelman portrayed the connection between the nuclear respiratory factors and mtTFA, which seems to definitively suggest that mitochondrial function in these thermogenic cells is achieved through the modulation and tuning of the two factors. Using cotransfection, the two researchers demonstrated that PGC-1 expression (within a population of BALB 3T3 cells in culture) was able to amplify the activity of the mtTFA promoter region by 300% (Cohen & Spiegelman, 2015). However, what was even more interesting about Cohen & Spiegelman’s work, was their observation that a purposefully introduced mutation in the NRF regions completely negated this amplification. In theory, this likely means that PGC-1 may act at the transcriptional level, or possibly by affecting the expression of nuclear respiratory factors. Furthermore, it is of course possible that the influence occurs through a mixture of both mtTFA transcriptional activity and NRF expression.

The mRNA diagnostics for the two scenarios (mtTFA/NRF) were performed by Cohen & Spiegelman in 2014, and it does appear that myoblast/myotube cells with forced expression of the PGC-1 increase both NRF-1 and NRF-2 mRNA, along with more real-time expression and content of mtTFA (the target gene) (Cohen & Spiegelman, 2015). Other relationships outside of cold-induced mitochondrial proliferation have been previously identified amidst PGC-1 and NRFs, including, but not limited to, several roles
in hormonal responses and cytochrome oxidase activity (Venditti et al., 2009). In summary, while the relationship between thermogenic proteins and mitochondrial activities is becoming increasingly defined, there are still many questions and undefined relationships that are of ongoing interest.

DISCUSSION

Overview

One of the largest ongoing debates in the field of thermogenic fat research involves whether or not brown and/or beige fat is a practical route to improve a patient’s metabolic health. Before such a practicality can be explored, scientists have spent the last decade collecting information on how brown fat is activated, where it is found, and how to identify it. Though this last aspect of identification may seem like the most straightforward issue, it is surprisingly somewhat of a burden, as the amalgamation of brown cells within white tissue deposits necessitates the use of expensive CT and PET imaging scans, which are not cost-effective measures when used for human analysis.

Nevertheless, as less invasive and more economical methods of brown fat identification are explored, the attention likely shifts into the use of novel therapeutic agents that may be able to trigger this brown and beige fat. By contributing to a patient’s basal metabolism, this creates a means to lose additional weight through a negative energy balance, and consequently forms a buffer against weight-gain—and hence obesity. This concept is especially recent, with the 2015 work of Dr. Aaron Cypess and the
mirabegron pill treatment for overactive bladders. This oral regimen, which acts as an agonist for beta 3 adrenergic receptors, appears to simultaneously stimulate BAT through the same receptor-mediated mechanisms.

FDG-PET scans confirmed the actions of the mirabegron product, raising the metabolic rate of twelve patients by an average of 203 +/- 40 kcal/day: approximately a 13% average increase (Cypess et al., 2015). Although the long-term consequences (i.e. side-effects) of thermogenic recruitment via the sympathetic system are largely unknown, such strong clinical performance—despite the small sample size—is promising in terms of the future of pharmaceutical interventions using this approach. Additionally, Cypess et al.’s findings show that such a medication may be extremely helpful in combatting fatty liver diseases, which is particularly relevant when considering the implications for liver transplant lists in the United States.

**Countering the Pessimist’s View**

Opponents to the relevance of thermogenic depots as a viable means for weight loss are quick to cite disparities between UCP-1 mRNA and their respective (brown or beige) sources. While beige/brite fat shows a remarkable increase (more than 200-fold) in UCP-1 protein product when cold-thermogenesis is triggered, authors like Jan Nedergaard have hypothesized that this dramatic increase is not so much attributed to a physiological significance, but rather a product of exceedingly low basal levels prior to the thermogenesis (Nedergaard & Cannon, 2013). Nedergaard and Cannon’s 2013 article “UCP1 mRNA does not produce heat” suggests that brite/beige fat may constitute less
than one-tenth of classical brown tissue depots, meaning that the UCP-1 mRNA is not an appropriate proxy for thermogenic contributions to body metabolism.

While Nedergaard and Cannon do raise legitimate points concerning the tendency of the scientific community to recently downplay classical brown fat in favor of the beige/brite hybrid tissue, ultimately scientists must rely on some form of proxy when attempting to quantify an intangible physiological process (i.e. heat produced solely from a hidden subpopulation of cells). Therefore it is not entirely unreasonable to use UCP-1 to collect valuable end-points. More importantly, opponents also fail to suggest an alternative method for collecting data on these two tissue-types, which is a major flaw in this Nedergaard and Cannon criticism. In fact, the recent work on mirabegron patients by Cypess seems to diminish the importance of defining the “segregation of labor” between the two forms of thermogenic fat, seeing as the results are paramount.

Naturally, this leads into the biggest controversial subject, which is best summarized by the question: “do humans have enough thermogenic capacity to make a difference?” While it is certainly conceivable that rodents do possess a much greater thermogenic capacity when standardized for personal body volume, it must be noted that given the gravity of obesity-associated diseases in the United States, even small pharmaceutical interventions—if proven safe for use—would be extremely effective when used in combination with proper diet and exercise.

In essence, every kilocalorie counts, and therefore even small energy deficits over a large span of time would inevitably yield tremendous results in the patient population. While only 100 kilocalories of increased metabolism per day may seem insignificant
when dealing with patients who are perhaps more than 100 pounds over their desired clinical weight, the accruing nature of a caloric deficit means that consistent treatment

A Multimodal Approach?

It is conceivable that future regimens may not merely be confined to one form of therapy, and therefore even small efficacies (for example, a 5% increase in basal metabolic rate) have tremendous clinical significance for patients who may also be supplemented with either a novel treatment for appetite management or a SFLT2 inhibitor such as sergliflozin, which rids the body of glucose. If these theoretical treatment options become realities, clinicians may be well positioned to help patients counter their weight problems, assuming that these treatments do not also introduce additional risks for the patient.

At this point in the battle against obesity and Type-II Diabetes, there have yet to be significant longitudinal studies that track the undesired interactions of drugs like mirabegron on other parts of the human body. However, in the next ten to fifteen years, there will undoubtedly be much more data on these interactions, and that information will ultimately determine the sustainability of such a treatment.

Lingering Questions

Aside from the clinical aspect, it is certainly plausible that the research-side of thermogenic fat is still in its true infancy. Sizeable questions remain: Is it possible that thermogenic fat is further subdivided beyond merely brown and beige? What is the origin of brown, visceral fat that does not seem to share a Myf-5 lineage? As researchers
become more acquainted with the progenitors of these tissues, it is likely that more routes will emerge through which to “hack” the biochemical processes that directly influence a patient’s metabolism. Additionally, in the next ten years, it is likely that scientists will better-characterize the anti-diabetic effects of thermogenic fat, possibly connecting the incidence of improved glucose-tolerance with other systemic regulators of body metabolism.

For thermogenic-therapy advocates, the race to introduce a safe and reliable therapy is likely coinciding with the quest by other researchers who are actively trying to develop ways to manage appetite through the neural orexigenic and anorexigenic systems. Although the sophisticated nature of our appetite signaling has created many hurdles for those who are attempting to create a negative energy balance by reducing food consumption, it may only be a matter of time before someone manages to create a successful form of appetite-based treatment. If such a therapy were to encompass a continuum by which physicians could closely monitor and subsequently tailor the dosages to avoid excessive fatigue or malnutrition, it would likely be preferred over a thermogenic fat avenue, as it would carry a much higher ceiling for rapid weight loss.

**Prediction**

In the next ten years, it should be expected that more sizeable advancements will be reached in the battle against obesity and Type-II Diabetes. The “Diabetes Belt” alone, comprised of 15 states in the Southeastern United States, now carries a population in which over 11% of the inhabitants are diagnosed with diabetes (Barker et al., 2011). These figures are continuing to expand on an annual basis, placing even more stress on
our healthcare system, and driving the total cost (includes indirect expenses) of delivering affordable healthcare to this patient subset to upwards of $250 billion per annum. These statistics undoubtedly create a collective incentive to address the trends, through a combination of diet, exercise, and medical interventions. With a target market of such magnitude, both large and small pharmaceutical entities are heavily invested in developing targeted therapies for obesity.

In addition, within the context of thermogenic fat exploitation, one intriguing aspect of the quest for a targeted protein therapeutic (or uncoupling agonists in general) revolves around the concept of relative risks. Generally, when new therapies are created, they must not only be effective, but must also carry benefits that significantly outweigh any undesired side effects or health-risks associated with the treatment group. However, what makes obesity so unique, is that the highly tangible risks of excess adipose tissue create a larger margin-for-error when experimenting with a new treatment.

Table 1 (Below) shows the relative risk of several health conditions in obese males, collected over multiple years from a sample of 6,987 patients. When comparing normal-weight males alongside males diagnosed with Class 1 Obesity (defined as a low-risk patient whose BMI is between 30 and 35) it becomes evident how dangerous a state of hyperadiposity is toward the human body. It should be noted that the risk of osteoarthritis and gallbladder dysfunctions appears to worsen rather dramatically as Obesity Class increases in severity, more so than the other four diseases. This either suggests a special sensitivity of these diseases to excess fat storage, or may demonstrate
<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Relative Risk vs. Normal Male</th>
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<tr>
<td>(In Patients with Class I Obesity)</td>
<td></td>
</tr>
<tr>
<td>Diabetes (Type II)</td>
<td>+4.98 x</td>
</tr>
<tr>
<td>Gallbladder Disease</td>
<td>+2.79 x</td>
</tr>
<tr>
<td>High Blood Pressure</td>
<td>+2.09 x</td>
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<tr>
<td>Coronary Heart Disease</td>
<td>+1.81 x</td>
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<td>Osteoarthritis</td>
<td>+1.80 x</td>
</tr>
<tr>
<td>High Blood Cholesterol</td>
<td>+1.47 x</td>
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</tbody>
</table>

Table 1: Relative Prevalence Ratios for Obese vs. Non-Obese Males, sorted by relative risk value. Public data from the Third National Health and Nutrition Examination Survey (1994) was analyzed in Microsoft Excel. Prevalence ratios were higher in all categories for obese males and females. Type II Diabetes, Gallbladder Disease, and High Blood Pressure were well-over twice as common in Class I patients, when compared against “normal” patients with normal BMI’s (normal range:18.5-24.9 kg/m²).
how even a slight amount of excess fat may incur serious consequences on the human physiology.

There is tremendous gravity behind the figures shown above in Table 1, as it quantifies the extent to which excess adiposity trickles into other areas of medicine. It is clear that the medical community as a whole would greatly benefit from a successful new amendment to fat-loss; therefore it is reasonable to believe that with 1) added public incentives, 2) advancing imaging and diagnostic technologies, and 3) time, the next ten years of scientific inquiry will include a deeper understanding of thermogenic fat, and any undetermined “cross-talk” and/or interplay with other components of the body’s metabolism.

Unintended Consequences of Future Developments

Previous visions of a “fat pill” or an “imaginary meal” should not necessarily be cast aside altogether, however there are many disadvantages in terms of using medications that may trigger undesirable behavioral changes in patients, including complicated rebound effects and other serious neurological events like addiction. Rebound effects have been a bane for non-thermogenic weight-loss and diet suppression-centered therapies, and thus far, studies of cold-induced thermogenesis have not illustrated this phenomenon in mice models. Conversely, an “imaginary meal” pill, if abused by patients, may diminish the potential benefits of the weight loss, creating a scenario where malnutrition and gastrointestinal irregularities necessitate additional (and more acute) medical interventions. For these reasons, the exploitation of thermogenic fat
is a superior route to address the issues, given both the current understanding of its safety, distribution, and relative compartmentalization of the active tissue.

**Forward Remarks**

An ideal pharmacological treatment for obesity undoubtedly will recruit a proper combination of diet and exercise. Though recent clinical trials have demonstrated that placebo-matched studies are effective during treatment periods, weight gain often resumes when a patient discontinues the regimen. This is truly a testament to how a pill-driven approach may not be sustainable without significant breakthroughs in the neurological synapses that govern appetite and the reward system. The chronic nature of obesity is such that patients must make a commitment to monitoring their dietary and exercise habits, or else succumb to a perpetual course of medications, which may have side effects that can only be captured during post-marketing vigilance periods.

Given the magnitude of how many net kilocalories must be burned to remove fat from the body, as well as the sheer mass of excess adipose tissue on obese patients, current treatment options are currently confined to long-term monotherapies. This horizon additionally creates the potential for undesirable interactions, and necessitates contraindications for patients who have a myriad of circumstances including pregnancy, cardiovascular disease, hypertension, and MAOI use (Yanovski, 2014). In addition, considering the associations between overweight individuals and the likelihood of these patients fitting criteria for a contraindication, this greatly limits the safety and benefit of the therapy.
Of the current FDA-approved obesity medications, only Phentermine/Topiramate-ER, Lorcaserin, and Orlistat are acceptable to administer over a prolonged period exceeding multiple weeks. However, despite limited efficacy when compared against trial placebos, these three drugs have a significant list of common adverse effects, which suggests that the future of weight management likely does not involve an appetite suppression model. Across these three suppression-based drugs, gastrointestinal and neurological side effects are rampant, particularly for Lorcaserin: a 5-HT2C receptor agonist that places patients at risk of hallucinations and neuroleptic syndrome (Yanovski, 2014).

Considering the high medication costs, (exceeding $200 per month) Lorcaserin (marketed as Belviq) does not provide patients with an excellent return on their investment, and therefore there is currently an incredible opportunity to create a novel therapeutic that will serve a broader population with fewer contraindications and more overall efficacy. Table 2 (Below) shows a categorical analysis of the three long-term orexigenic suppressants. (Lorcaserin, Orlistat, and Phentermine/Topiramate-ER)

Over the course of many obesity-related longitudinal studies, there exists a common theme wherein overweight children are far more likely to remain obese into adulthood than to regress to a mean population BMI after adolescence (Clarke et al., 1993). Taking this into account, perhaps the future of pharmaceutical intervention should involve battling obesity not after the damage has occurred, but rather while young patients have not accrued too much fat, and are still able to boost their exercise habits before excess weight becomes a burden for movement and energy expenditure. Although
<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Cost/Month (in USD)</th>
<th>Mean Weight Change (in lbs/year)</th>
<th>Mean Cost (per pound lost)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phentermine/Topiramate-ER</td>
<td>195</td>
<td>-19.6</td>
<td>$119.39</td>
</tr>
<tr>
<td>Orlistat</td>
<td>207</td>
<td>-7.5</td>
<td>$331.20</td>
</tr>
<tr>
<td>Lorcaserin</td>
<td>240</td>
<td>-7.1</td>
<td>$405.63</td>
</tr>
</tbody>
</table>

**Table 2: Costs of Approved Appetite-Suppressing Drugs**, Data taken from “Long-term Drug Treatment for Obesity: A Systematic and Clinical Review,” Yanovski & Yanovski, 2014, and analyzed using Microsoft Excel. Estimated monthly costs for the drug were taken from the high end of the patient pocket costs, using 2013 U.S. dollar values. Weight changes were pulled from the highest commonly prescribed dosage for each drug. This table illustrates the expensive nature of appetite suppressing medications, which show fairly minor clinical effectiveness when compared to placebo-receiving individuals. In the context of future thermogenic therapeutics, it appears that a drug priced below $400 (per pound lost, per year) would be extremely competitive when compared with these long-term medications, which have undesirable systemic side effects.
administering such a proactive biopharmaceutical could pose a threat to uncharacterized steps of adolescent development, an argument could be made that the risks of hyperadiposity far outweigh any mild risks that may be associated with such a regimen. Therefore it is imperative that medical scientists dedicate more resources into preferentially approaching this younger target demographic, to relieve the ever-growing load on the U.S. healthcare system.

**Concerns for Future Study**

Likewise, a shift into more preventative options mandates a thorough understanding of the brown/beige fat life cycles, a grasp of where these cells truly originate from, as well as a precise theory of how and when genetically defined modifications in the thermogenic fat distribution occur. With recent investigations revealing that WAT presumably controls its total cell-count throughout the body, perhaps it is possible that there is a mechanism (analogous to the “gonadostat” in pubescent males and females) that is responsible for controlling how the body will handle its adipose stores for the post-pubescent state. In the advent of the beige/brite discovery, it would make sense for there be a physiological regulator of all of the fat stores, perhaps bridging fat thermogenesis with the control of white adipose distributions. For these reasons, it would be prudent to investigate how pre-pubescent mammalian fat distributions affect the full-grown status in-vivo, looking for targets that could potentially be administered to humans in order to influence adult adipose characteristics.

The population trends for Obesity and Type II Diabetes in the United States are incredibly disturbing. According to the most recent data from the 2013 Behavioral Risk
Factor Surveillance Systems (BRFSS) division of the Centers for Disease Control and Prevention (CDC), no state had a self-reported obesity (BMI > 30) prevalence of less than 20%. In addition, for the first time in the history of this survey, two states (West Virginia and Mississippi) are believed to exceed 35%. Both the South and Midwest regions had the highest prevalence (30.2% and 30.1% respectively), followed by the Northeast (26.5%) and West (24.9%) (CDC, 2014). In terms of ethnicity, African Americans had a 37.6% self-reported prevalence, significantly higher than Hispanics (30.6%) or Caucasians (26.6%) (CDC, 2015). As thermogenic fat research becomes more advanced in human subjects, it may be advisable to study genetic differences between different ethnic groups, to determine whether or not thermogenic fat varies significantly in these patients. In the coming years, it will be interesting to see if mirabegron (the over-active bladder medication that was recently found to induce thermogenesis in humans) is preferentially effective among different subpopulations.

**Figure 6** (Below) and **Figure 7** (Below) collectively establish how fast the obesity epidemic has evolved in the United States. In 1993, it is believed that no state exceeded 20% in terms of self-reported obesity (after adjusting for changes in survey techniques). However, by 2013, Colorado was the “leanest” state, at 21.3% self-reported obesity.
Figure 6 (1994) establishes a standard for comparison, using data gathered in 1993, two decades before the information was collected in Figure 9 (See below). Both figures were made using public data from the CDC’s annual obesity status report. It is possible that this data set underrepresents the true prevalence of diabetes in the continental U.S. at the time, as polling techniques were later changed to reflect the use of cellular telephones. No data for Wyoming was collected; however, given the information on surrounding northwestern states, it is a reasonable assumption to believe that the state would fit in the 10-15% range.
Figure 7: (2014) Twenty years later, this prevalence map illustrates the serious nature of obesity in the United States. If this trend continues into 2020 and beyond, healthcare costs will continue to grow linearly, compromising both the health of average Americans, as well as the ability of current physicians to efficiently handle the broad spectrum of diseases that are associated with obesity. By 2023, several more states (in addition to Mississippi and West Virginia) will likely exceed 35% self-reported obesity.
Conclusion

The impact of obesity on the broader population has already started compromising healthcare considerably, and unless some serious changes are made, inevitably these key issues will continue to expand uncontrollably. Despite a widespread notion that childhood obesity has tremendous negative physical and psychological repercussions, addressing this serious disease remains relatively low on the priorities of U.S. policymakers. A more thorough investigation of treatment options, specifically through preventative measures is not merely a “just” response, but rather an essential action that must be initiated immediately.

There are two theoretical routes by which thermogenic fat could be exploited in humans to create a sustainable negative energy balance in overweight patients. First, there is an in-vivo avenue, whereby medical scientists will seek to use small proteins and known factors that affect BAT differentiation and growth. The advantages of this approach include the extent to which it was proven effective in the studies of clinical DNP use. However, there are several risks associated with this avenue, chiefly the concept whereby the brown adipogenesis yields unintended consequences when employed in the body. Furthermore, the exact origins of the precursor cells are not completely defined, and therefore it may several more years before this approach is available for safe human exploration.

The second avenue seeks to increase thermogenic-uncoupling activity in patients, theoretically lowering the amount of physical exercise that is required to maintain a negative energy balance. Of the two options, this particular intervention in BAT-
Figure 8: Two Approaches for Thermogenic Therapy: Visual created by Robert D. Stibolt Jr. for the purposes of this thesis. This flow-chart includes the most widely accepted targets for future thermogenic interventions. For the Ex-Vivo approach, the initial progenitors derive from Mesenchymal Stem Cells.
mediated metabolic energy expenditure may have a higher ceiling in terms of its therapeutic potential in the clinical setting. Moreover, this route may be accomplished ex-vivo, by growing cells in-vitro and subsequently transplanting them back into the original donor. While the ceiling may be higher for this second method, this also commands more potential risks for patients. A possible unintended consequence includes stimulating a patient’s appetite, and therefore requiring even more energy expenditure to offset the additional intake. In addition, there are always associated risks when inserting cells into the body, though the localized nature of the thermogenic reservoirs may be less conducive to creating mobile tumors. **Figure 8** (above) explains these two routes in a more visual manner.

With the obesity epidemic sweeping through the United States, it is clear that clinicians are in need of novel approaches to counter weight gain. In the last five years, knowledge of thermogenic adipose tissues has been amplified tremendously, and this will likely be the primary means by which future scientists circumvent other obstacles that have previously arisen when seeking an appetite suppression-based therapy. Addressing childhood weight gain may be key in preventing the further expansion of obesity and type 2 diabetes; several decades ago, this segment was practically unaffected by these diseases, however over 25% of children in the United States now are considered either overweight or obese (Cypess & Kahn, 2010). Though it may be risky to pioneer a possible role of thermogenic fat in younger patients, it may be worth the associated risks, given the gravity of what obesity prescribes for long-term health.
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Research Assistant, August 2013-May 2014

• Worked in oncolytic virotherapy and vector production under Dr. Theresa Strong. Created cell-based assays for a Phase-I FDA investigational new drug project involving CRAd-S-pK7 virus.

VOLUNTEER WORK

Habitat for Humanity of Greater Birmingham
Construction Volunteer, June 2013-June 2014

• Weekend participation on projects that involved construction, renovation, and demolition of houses in disadvantaged neighborhoods. Duties included occasionally directing large volunteer groups on habitat builds.

Karl E. Alexander Foundation
Event Organizer, July 2010-December 2011

• Founded an annual event called “5K for Karl,” contributing over $20,000 toward an endowed memorial scholarship. Garnered recognition including the Wofford College IFC 2011 Best Philanthropy Award, and Kappa Sigma Fraternity 2011 Distinguished Philanthropy Award.
SKILLS

• **Laboratory**- cell culture, cell staining and counting, viral infection, differential cell killing, statistical analysis of colorimetric assays, daily laboratory upkeep, inventories, lab safety.

• **Leadership**- event organizing, team meetings, regional and national representation, goal planning.

• **Technology**- Adobe Photoshop, Filmmaking, Microsoft Office, Database Creation, Financial Reporting Programs.

AWARDS

2013 • Wofford College Dean’s List, Cum Laude Graduate

2012 • Alpha Epsilon Delta – Health Preprofessional Honor Society, Kappa Sigma Scholarship Leadership Award, Wofford College Dean’s List

2011 • SCICU Research Grant Recipient