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Novel Role for a Neurotrophic Factor in White Adipose Tissue

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NOVEL ROLE FOR A NEUROTROPHIC FACTOR

IN WHITE ADIPOSE TISSUE

by

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Abstract

Neurotrophic factors are a family of growth factors that regulate neuronal plasticity. Thus far, these factors have been understudied in peripheral tissues, including adipose tissues, where they could play a key role in mediating the neuronal inputs that lead to energy expenditure via lipolysis (white fat) and thermogenesis (brown fat). Based on prior experiments, we hypothesized that brain derived neurotrophic factor (BDNF) is the main neurotrophic factor acting in adipose tissues to mediate neurite outgrowth and branching of incoming sympathetic nerves. We found that BDNF knock-out animals had less innervation of their white fat, shown by reduced expression of neuronal markers, but a paradoxical increase in cold-stimulated brown adipogenesis in white fat ('browning'). Further investigation using immunofluorescent staining indicates that although browning can be activated by some alternate factor that remains ambigious, innervation and stimulation of the sympathetic nervous system is required for activation of UCP1-mediated thermogenesis.

Acknowledgements

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Abbreviations

CDC	Center for Disease Control
WAT	White Adipose Tissue
Th 1	Type 1 T helper cell
BAT	Brown Adipose Tissue
UCP1	Uncoupling Protein 1
MRI	Magnetic resonance imaging
PET-CT	Position emission tomography-computed tomography
ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
BDNF	Brain Derived Neurotrophic Factor
TrkB	Tropomyosin-sensitive receptor kinase B
MAPK	Mitogen-activated protein kinase
P13K	Phosphatidylinositol 3-kinase
PLCγ	Phospholipase Cγ
Lys-M	Lysin Motif domain
KO	Knock-out
Con	Control
PBS	Phosphate buffered saline
EtOH	Ethanol
ORO	Oil Red O
VEGF	Vascular Endothelial Growth Factor

Introduction

In 2013, the Center for Disease Control (CDC) estimated that 69 million people were considered obese in the United States alone (The World Health Organization, 2016). Obesity and the metabolic diseases that are associated with obesity are increasing at an alarming rate due to the modern day high calorie diet and sedentary lifestyle as well as genetic predisposition. Obesity is accompanied by an increased risk of developing both mechanical and physiological comorbidities that are serious, non-reversible and often deadly. These comorbidities include sleep apnea, osteopathic complications, cardiovascular disease, hormone-related cancers, cardiovascular disease and insulin resistance, as seen in type II diabetes (Wilding et al., 2012).

The root of the obesity problem is an accumulation of adipose tissue resulting from improper energy balance between storage and expenditure. To understand and potentially correct this imbalance, it is first important to understand the mechanisms. Energy balance in the body is in part managed by two types of adipose tissue (white and brown) as well as the systems that interact with them, including the digestive, vascular and nervous systems.

White adipose tissue (WAT) stores lipid in large triglyceride droplets, which can be broken down to create energy when the process of lipolysis is initiated by neuronal stimulation. In cases of abnormally high amounts of lipid, adipocytes attempting to accommodate the needed storage can become very large, or hypertrophic. These large cells have a low surface area to volume ratio of the cells, which can inhibit diffusion of oxygen and important nutrients provided by vasculature and extracellular matrix which are needed for processes inside the cell. Without proper diffusion, the cell can become

hypoxic, or oxygen deficient, and die, leading macrophages (a type of phagocytic immune cell) of the adipose tissue to surround that cell and engulf it. Seen under a microscope, an image of this process is known as a crown-like structure (Murano et al., 2008).

Macrophages can be recruited to adipose tissue by chemotaxic signals emitted by the tissue in response to stress, such as cell death, hypoxia or cold exposure. Additionally, macrophages can be polarized to different physiological states and thus secrete different cytokines depending on the state of the tissue. Adipose-derived Th1 secretions in response to obesity cause macrophages to be polarized from their M2, anti-inflammatory state, to their M1, pro-inflammatory state, in which they secrete pro-inflammatory cytokines, creating an inflammatory and unhealthy state in the adipose tissue (Lumeng et al., 2007).

The other type of adipose tissue is brown adipose tissue (BAT) which functions to create heat by uncoupling oxidative phosphorylation from ATP production in a process called non-shivering thermogenesis, which is mediated by the BAT-specific uncoupling protein 1 (UCP1) (Cannon et al., 2004). In contrast to WAT, BAT is multilocular, meaning it has multiple small lipid droplets instead of a single large one (unilocular). BAT has a large number of mitochondria in comparison to WAT, which along with a dense vascular supply gives the tissue its characteristic brown color. BAT is present mostly in small mammals and the young of larger mammals; however, it has been shown that there are small amounts of BAT that can be detected by imaging like MRI or PET-CT in human adults (Nedergaard et al., 2007).

UCP1 (also known as thermogenin) is unique to BAT and crucial to its process of thermogenesis. UCP1 increases the permeability of the inner membrane of the numerous mitochondria in BAT, acting as a proton carrier and providing a route for protons/anions to pass through other than ATP synthase (Aquila et al., 1985). In normal mitochondrial function, a proton gradient is created by pumps in the inner membrane and protons pass through the membrane in order to satisfy the charge gradient that was created by the pumps (Camps et al., 1992). The enzyme ATP synthase allows the protons to pass through the inner membrane but in doing so, a separate subunit of the enzyme undergoes conformational changes which cause the enzyme to create adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Ko et al., 1999). By allowing the process of transmembrane proton transport to occur without the production of ATP by ATP synthase, the two processes are uncoupled. ATP is not created and the energy is dissipated as heat (Heaton et al., 1978). Because of this thermogenic role of BAT, the activation of UCP1-mediated thermogenesis in BAT causes a dramatic increase in glucose uptake and whole-body energy expenditure (Orava et al., 2011).

Although fat depots are primarily composed of brown or white fat, the formation of brown adipocytes, can occur in WAT through a process termed 'browning' with the help of innervation and vascularization in the area surrounding the tissue (Cousin et al., 1992). The new adipocytes are known by many different names including beige (Harms et al., 2013), brite (Petrovic et al., 2010), inducible (Lee et al., 2011), or recruitable (Schulz et al., 2013). The process termed 'browning' can be activated as a result of cold exposure (Barbatelli et al., 2010) and in mammals is common in young as a way to regulate body temperature. This process of browning serves as a natural way to shift the

energy balance back towards expenditure without an increase in physical activity or basal metabolic rate.

Innervation of adipose tissue is extremely important to the regulation of energy balance. In WAT, innervation and neuronal activation can stimulate lipolysis or the hydrolysis of triglycerides into glycerol and free fatty acids for use in creating energy through the process of cellular respiration which occurs throughout the body. As the lipid inside adipocytes is broken down and transported to other tissues, the cell size shrinks, leading to an overall healthier tissue. Innervation of WAT can also cause the creation of new adipocytes through adipogenesis, which is known as hyperplagia (Faust et al., 1978). In BAT, innervation and neuronal activation is thought to turn on UCP1-mediated thermogenesis. Since UCP1 function is activated by the binding of free fatty acids, innervation and subsequent lipolysis of WAT, which releases free fatty acids into circulation can also affect the level of thermogenesis. Furthermore, testing the effect of manipulating adrenergic agents and adrenoreceptors has also shown that sympathetic innervation is important in initiating browning of WAT (Nedergaard et al., 2014).

Neuronal health and plasticity throughout the body is largely dependent on a group of growth factors called neurotrophic factors. Neurotrophic factors are secreted by a developing neuron's target tissue and are responsible for the growth, plasticity and survival of those neurons by mediating the creation of new neurons (neurogenesis), creation of new synapses (synaptogenesis), and neuronal branching (Strand et al., 1991). The implications of neurotrophic factors in the brain and in the nervous system overall are evident. Less explored, however, are the implications of those same neurotrophic factors in adipose tissue.

The neurotrophic factor of interest in this study is known as Brain Derived Neurotrophic Factor (BDNF) and was first discovered in 1982 as the second member of the neurotrophic factor family (Barde et al., 1982). BDNF was first isolated from pig brain and used to support the growth and in vitro survival of embryonic chick sensory neurons (Leibrock et al., 1989). There is growing evidence that BDNF is crucial not only to life, but also metabolic health. It has been shown that BDNF homozygous knockouts (-/-) do not survive for longer than three weeks (Lyons et al., 1999). BDNF heterozygous knockouts (+/-) as well as mice with post-natal BDNF gene deletion are prone to obesity (Kernie et al., 2000, Rios et al,. 2001).

A possible BDNF mechanism has been uncovered in which BDNF binds with its high affinity receptor Trk-B, a member of the family of tropomyosin sensitive receptor kinases (Kaplan et al., 1991, Klein et al., 1991). When the ligand BDNF binds to the full length TrkB isoform, homodimerization initiates cross tyrosine phosphorylation in intracellular TrkB domains and initiate one or more intracellular signaling pathways (Yoshii et al., 2009). These pathways include mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K) and phospholipase Cγ (PLCγ), which work to increase dendritic spine density and plasticity (Reichardt et al., 2006).

The Townsend Lab at the University of Maine is interested in unveiling the role that the nervous system plays in metabolic health and understanding the mechanisms which can be manipulated to improve metabolic health. In the preliminary stages of this collaborative study, Townsend found that secretions of the neurotrophic factor BDNF were increased in subcutaneous white fat in response to cold exposure. Fluorescence activated cell sorting was used to determine that the BNDF source was not mature

adipocytes, but Lys-M myeloid lineage cells present in the stromal vascular fraction, including the previously mentioned macrophages involved in the adipose inflammatory response.

Therefore, to investigate the importance of the neurotrophic factor, BDNF on peripheral nerve activity and implications in adipose tissue, Cre-Lox technology was used to create a mouse strain with a tissue specific knock-out. LysM-Cre mice, which express Cre recombinase only in a subset of immune cells, including macrophages, were mated to BDNF-floxed mice, leading to BDNF deletion in LysM cells.

The hypothesis for this project was that KO mice would have decreased peripheral nerve recruitment and function in adipose tissue and therefore inhibited browning of WAT. Also, any recruitable brown adipocytes that did form were not expected to be able to initiate non-shivering thermogenesis. The implications of these outcomes would most likely result in an increased animal weight due to larger WAT depots as well as presence of adipose inflammation.

I, in collaboration with other members of the Townsend Lab, aimed to investigate the morphological changes in adipose tissue caused by the deletion of BDNF in this transgenic mouse model. Morphological changes were assessed as indicators of adipose depot health and presence of UCP1-protein indicated thermogenic function. My hope is that revealing the importance of BDNF to adipose health and energy balance will initiate further research into the therapeutic options of the neurotrophic factor for the prevention and treatment of obesity and its comorbidities.

Methods and Materials

Mice

All mice were products of mixed genetic background matings of LysM-Cre and BDNF-flox, both of which were purchased from Jackson Lab in Bar Harbor, Maine. Only male mice were used because in previous cohorts no difference in phenotype was observed and males would not exhibit variation due to the reproductive cycle. The 5 control animals were Cre -/- mated with lox+/lox+ and the 8 KO animals were Cre +/- mated with lox+/lox+.

Treatment: Cold Exposure

The mice were allowed to reach maturity and then acute cold exposed (4-day duration) in a specially designed Caron Diurnal Incubator on 5/1/15 immediately prior to terminal tissue collection on 5/5/15.

Adipose Tissue Dissection

Subcutaneous (flank) adipose depots were carefully dissected and weighed. Tissue was placed in a cassette and stored in 10% buffered formalin overnight and the next day moved to PBS (phosphate buffered saline) and later embedded in paraffin by the UMaine Histology Core.

Histology

Tissues were sliced using a microtome with a thickness of 5 μm and three consecutive sections were placed on a positively charged Superfrost slide, with a total of

six slides were made with serial sections for each animal. After slicing, slides were baked overnight in a 40 °C oven and then stored at room temperature.

Pre-staining Procedure

Before staining any slides, the tissues were first deparaffinized. Histochoice clearing agent was used followed by a series of washes in ethanol followed by a rinse in distilled water. Tissue were then stained or stored overnight in distilled water for staining the next day.

Hematoxylin Stain

Hematoxylin stain, which stains the nuclei, cytoplasm and extracellular matrix in the cell, was used to stain slide #3 from each animal in the cohort that was used for this experiment. To stain, de-paraffinized slides were soaked in hematoxylin stain for 3-4 minutes, then washed in distilled water. Slides were then de-stained in ammonia hydroxide and finally rinsed in distilled water for another 3 minutes, left out to dry and stored at room temperature.

Immunostaining

Dako reagent was first used for antigen unmasking (2X10min in the microwave on low) followed by a wash with Millipore rinse buffer (cat# 20845 dilute from 20X to 1X). Sudan Black (0.3% in 70 EtOH) was used to block for auto-fluorescence for 20 minutes at room temperature. A Pap Pen was used to make a hydrophobic barrier around each tissue to keep it hydrated. Millipore Block (cat # 20773) was used to block for non-

specific staining for 20 minutes at 37 °C. Primary antibody was then diluted in Dako antibody diluent and 100µl was added to each tissue. Slide #2's from each animal were treated with PGP9.5 Abcam at 1:1000 dilution. Slide #4's from each animal were treated with UCP1 Abcam at 1:50 dilution. Each experiment included one slide #6 as a control. After primary antibody was added to each tissue, the slides were kept in a humid chamber at 4°C for 24 hours.

The next day secondary antibody (Alexa 488 α Rabbit) was added for 10 minutes. Slides were kept in the dark for the remainder of the experiment (including this step) to prevent bleaching of the fluorophore.

The slides treated with PGP9.5 primary antibody were then stained with Oil Red O (ORO, cat # 01391 from Sigma, diluted to working solution and filtered with Watman Paper #1) which demonstrates the presence of fat or lipids. The slides treated with UCP1 were not treated with ORO to prevent any interference with the UCP1 stain. All tissues were covered with a small square coverslip and mounted with Millipore Mounting Media.

Fluorescent Scope Imaging

Pictures were taken on Nikon E400 which is capable of Brightfield, Hoffman Modulation, and 4-color fluorescence. Pictures were taken at 100x. The control slide with no primary antibody added was used to establish a level of background fluorescence and any signal above this level was considered significant.

Regardless of stain, entire tissue sections were carefully examined at 100 x total magnification in a systematic fashion in order to ensure that the entire tissue's

morphology was taken into account (see appendix A for protocol). Then, three representative images were taken at 100x total magnification and any interesting features, such as areas of browning, were photographed at 200x total magnification. Because I was the one running the experiment it was difficult to collect the data blindly. In order to prevent biased observations, I did not note whether the slide was from a KO or a control until after the photographs were taken.

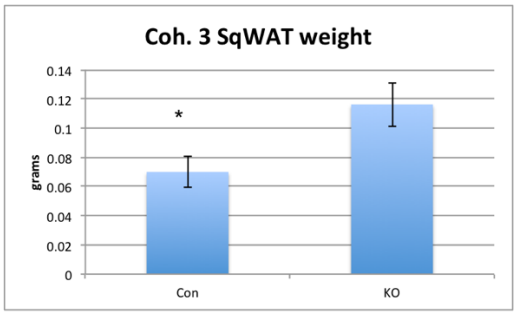
Analysis

Originally all microscope images were going to be analyzed through Image J imaging software in order to measure average cell size, which would have served as a measure for relative rates of lipolysis and an indicator of innervation and activation of the tissue. The Image J technology was not compatible with the images taken, so I relied on qualitative observations of cell size and used the amount of browning and crown-like structures present as an indicator of the tissue's innervation, activation and overall health.

Results

Preliminary Results:

A. *There is a trend for a lower body weight (BW) in Control animals and a statistically significant WAT weight.*



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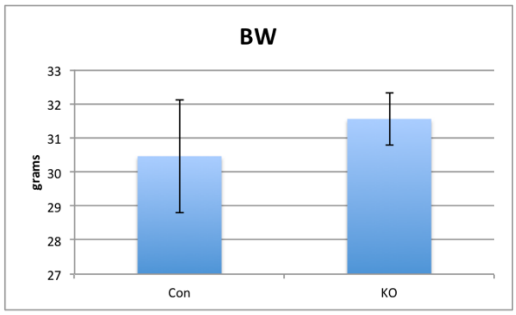
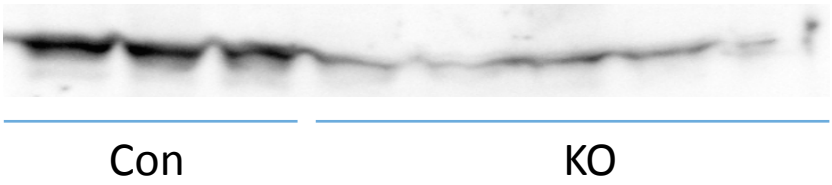


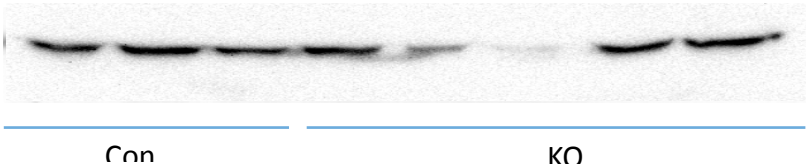
Not statistically significant

B. *KO of BDNF in LysM cells decreases the presence of innervation in adipose tissue*.

PGP9.5



β Tubulin



C. *KO of BDNF in LysM cells decreases the presence of innervation in adipose tissue*.

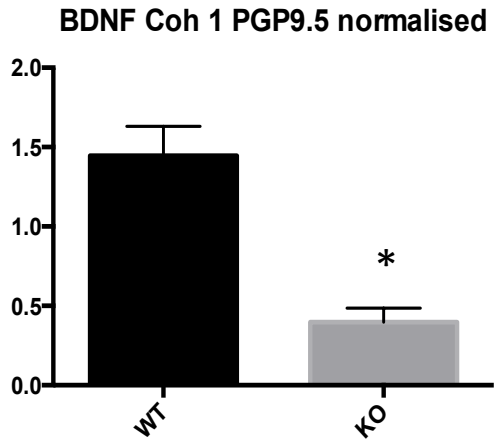


Figure 1. Preliminary Results (obtained by Magda Blaszkiewicz)
(A) Subcutaneous adipose tissue weights of control and KO mice compared to total body weight (BW)
(B) Western blot measuring presence of PGP9.5 in control and KO mice.
(C) Western Blot of PGP9.5 normalized and quantified.

Hematoxylin

Figure 2: *No significant difference in cell size or overall amount of browning was observed between KO mice and Control mice.*

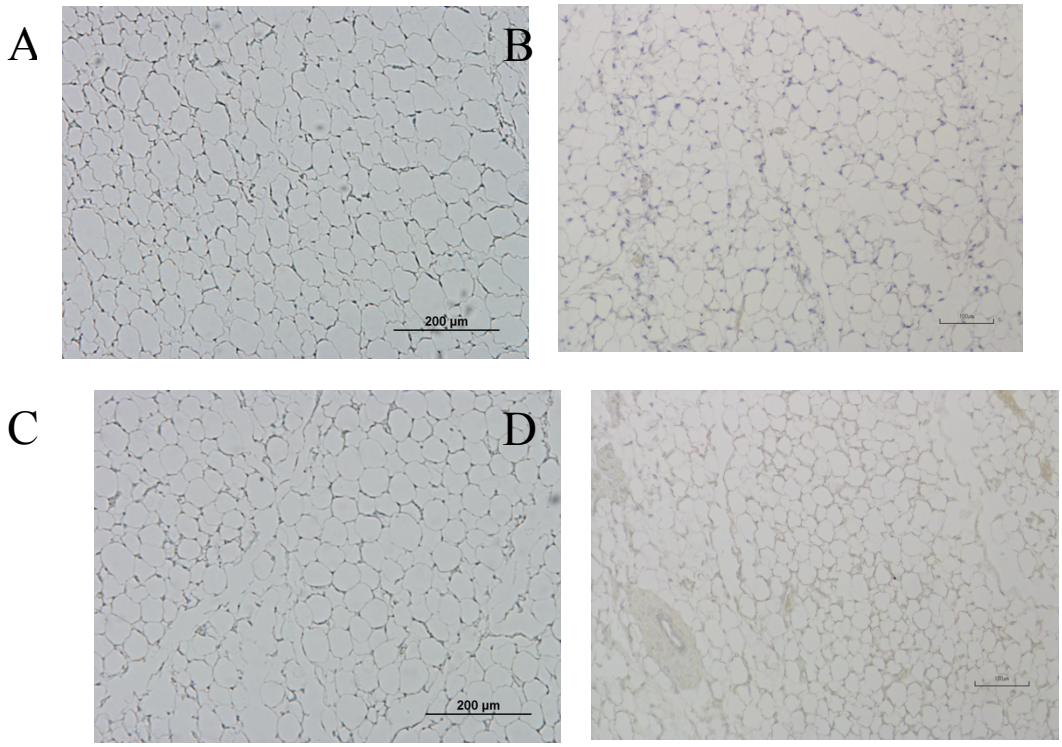


Figure 2. Representative images of average cell size using Hematoxylin stain.
(A) Representative Image taken at 100x of KO1 subcutaneous WAT with H&E stain.
(B) Representative Image taken at 100x of KO3 subcutaneous WAT with H&E stain.
(C) Representative Image taken at 100x of Con 1 subcutaneous WAT with H&E stain.
(D) Representative Image taken at 100x of Con 5 subcutaneous WAT with H&E stain.

Figure 3: *No significant difference in cell size or overall amount of browning was observed between KO mice and Control mice.*

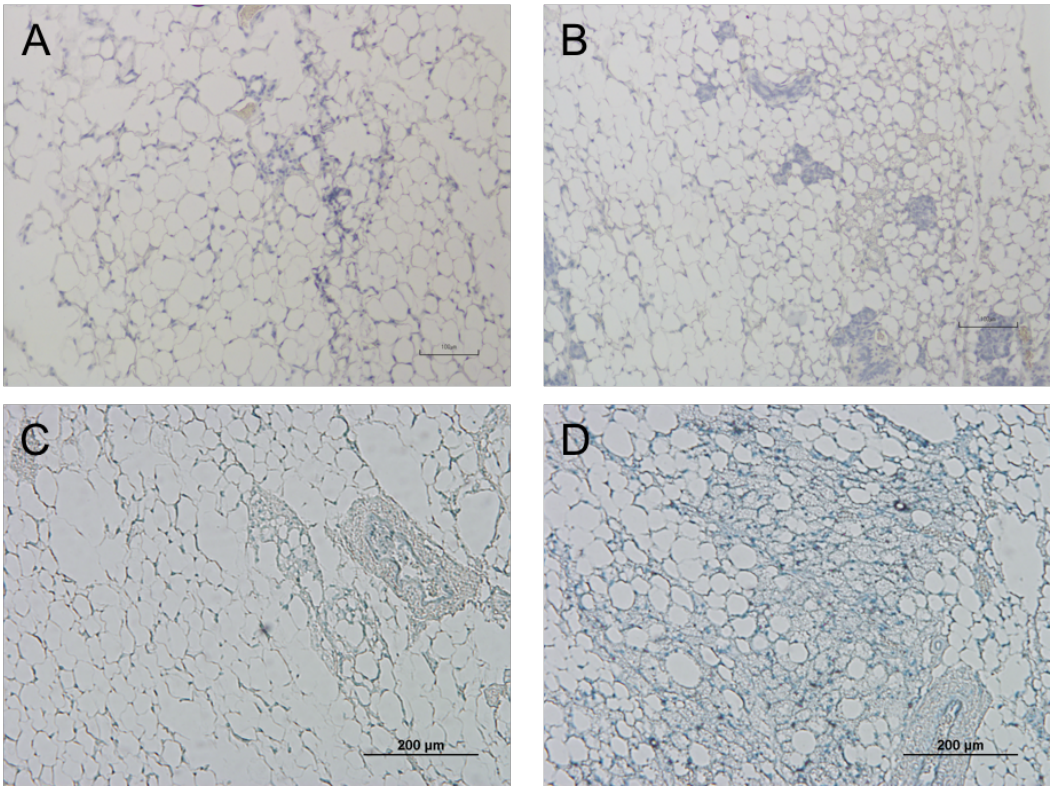


Figure 3. Images of browning in subcutaneous WAT.
(A) Microscope Image taken at 100x of Con 1 subcutaneous WAT with H&E stain.
(B) Microscope Image taken at 100x of Con 3 subcutaneous WAT with H&E stain.
(C) Microscope Image taken at 100x of KO1 subcutaneous WAT with H&E stain.
(D) Microscope Image taken at 100x of KO2 subcutaneous WAT with H&E stain.

Immunostaining: PGP9.5

Figure 4: L*ysM-BDNF KO mice express decreased innervation.*

A) Control 4 B) KO 7

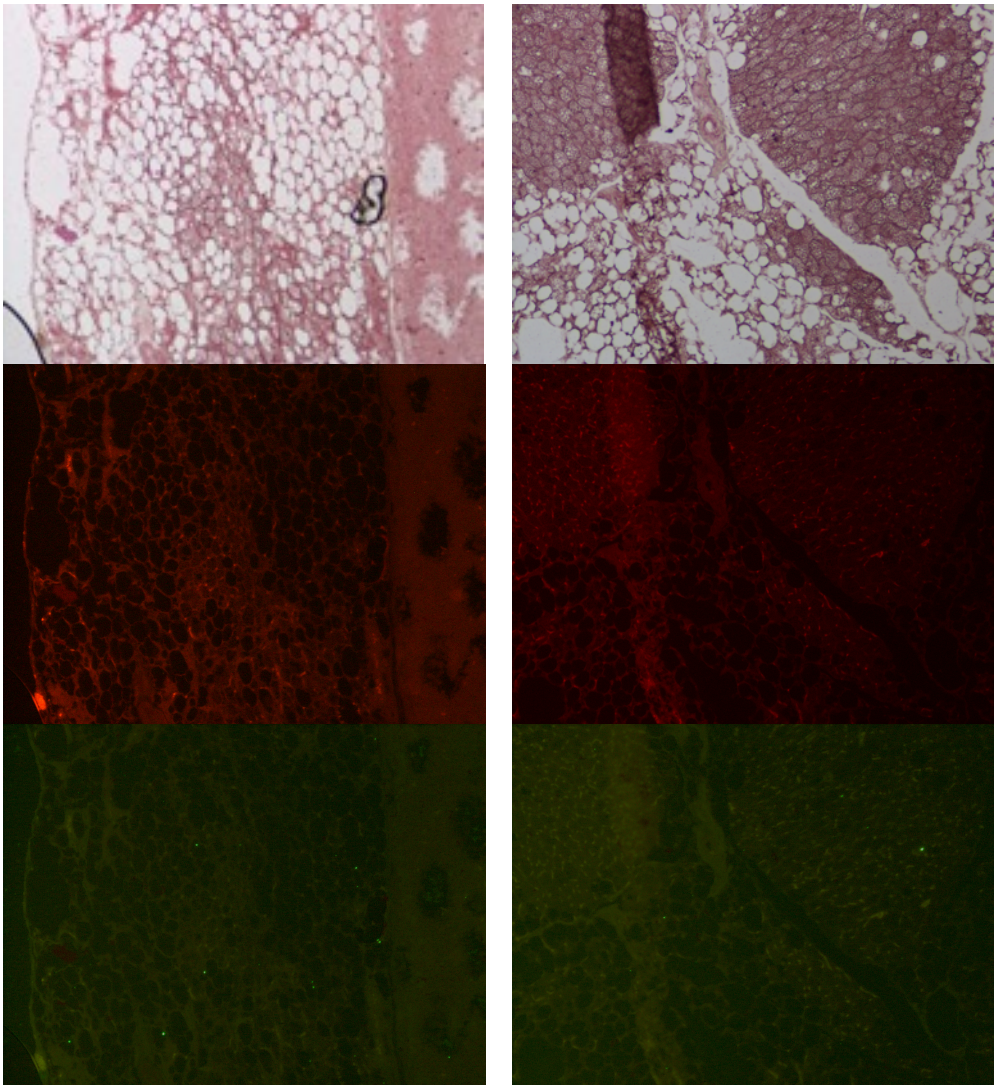


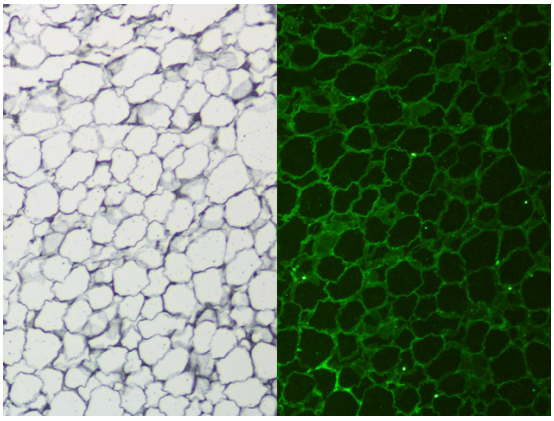
Figure 4. PGP9.5 Immunostaining: KO mice exhibit intense browning not matched by expected amount of innervation.

A) Mouse Con 4 immunofluorescent scope images. From top to bottom: Brightfield, ORO, PGP9.5 antibody.
B) Mouse KO7 immunofluorescent scope images. From top to bottom: Brightfield, ORO, PGP9.5 antibody.

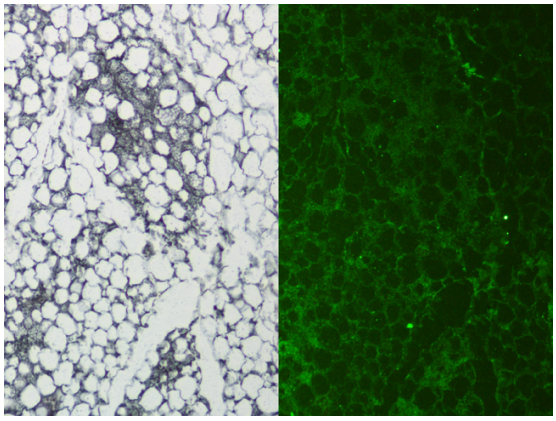
Immunostaining: UCP1

Figure 5: *KO mice lack expected UCP1 levels.*

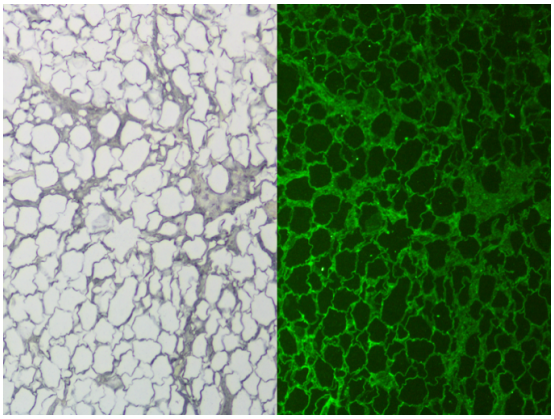
A) Control 2



B) KO 5



B) Control 5



D) KO 6

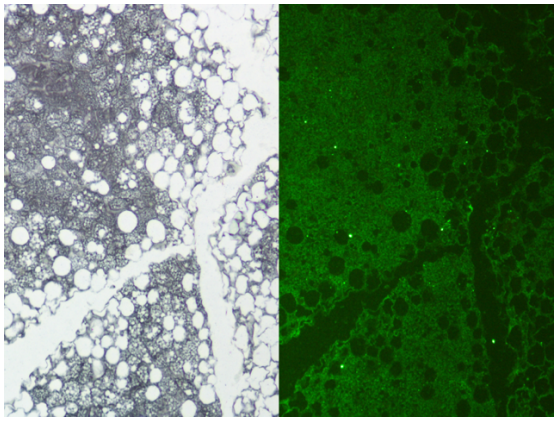


Figure 5. UCP1 immunostaining. KO mice exhibit dense patches of browning without expected brightness of UCP1 stain. Brightfield (left) and Immunoflourescent (right) images taken of slides stained with UCP1 antibody from the following animals:
(A) Control 2
(B) KO 5
(C) Control 5
(D) KO 6

KO of BDNF in LysM cells decreases the presence of innervation in adipose tissue.

To determine the effects of the neurotrophic factor BDNF, a mouse strain was created using Cre-Lox technology in which adipose LysM cells, including macrophages, with the ability to secrete the neurotrophic factor BDNF were genetically knocked out. This mouse strain was exposed to cold, which is a stress stimulus that is known to initiate browning of white adipose tissue and subsequent thermogenesis, so that the role and importance of BNDF in adipose metabolism may be better understood.

Initial results confirmed that genetically knocking out BDNF does hinder nerve growth and development. Both immunostaining and western blot with PGP9.5, an antibody to mark the presence of sympathetic nerves, indicated a lack of innervation in KO tissues. Protein levels in KO animals were significantly lower in the western blot (Figure 1C). Also, very dense areas of browning seen in KO mice were not accompanied by the expected amount of innervation that was previously thought to be crucial to the initiation of browning in WAT. Furthermore, control animals with little or no browning had a higher amount of innervation compared to those KO animals with large amounts of browning (Figure 4A-B).

KO animals exhibited large amounts of browning with no significant difference in cell size.

Multiple histology techniques were used to assess the morphologies of the adipose depots in this study. In order to consider cell size and amount of browning within the adipose depots, slides were stained with hematoxylin. Initial observations indicated an unexpected amount of browning in the WAT of the KO mice. Often, intense

browning was located adjacent to vascularization in the tissue section (Figure 3 C-D). This was accompanied by the observation that although there was more intense browning in KO animals compared to controls, there was no obvious difference in cell size between the two groups (Figure 2), which was unexpected since BAT is known to consume a large amount of free fatty acids in thermogenesis and the lipolysis required to supply those free fatty acids would logically result in a smaller cell size. The preliminary finding that KO animals had significantly higher subcutaneous (flank) weight compared to control animals (Figure 1A) indicates that there is a disconnect between the level of browning observed in the KO WAT and the expected lipolysis and burning of lipid that usually accompanies the presence of that browning.

KO mice lack expected UCP1 levels.

UCP1 activation is necessary for the process of thermogenesis in BAT. In order to measure UCP1 levels in the WAT, immunostaining with UCP1 antibody was performed. While the areas of browning as well as the unilocular traditional WAT areas of the control animals indicated the presence of UCP1, even the very dense regions of exhibited a comparatively low levels of UCP1 (Figure 5). Because the activation of UCP1 is necessary for the proper function of BAT in its role of thermogenesis, these findings indicate that the presence of neuronal stimulation, which is subsequently dependent on the presence of LysM cell secreted BDNF, is crucial to the activation of the UCP1-moderated thermogenesis in BAT; however, it is not needed for the initial browning of WAT, which was previously hypothesized.

Discussion

Adipose health contributes significantly to whole-body energy balance and if it is not properly managed, can lead to metabolic diseases with dangerous comorbidities such as heart disease and type II diabetes. The current study reveals a novel distinction in the understanding of adipose tissue mechanisms between what appears to be the source of initiation of browning in WAT and activation of UCP1-mediated thermogenesis in those recruitable BAT cells.

Fundamentally, due to the lack of PGP9.5 marking in LysM-BNDF KO mice which was seen both in the PGP9.5 western blot and immunostaining, this study suggests that the LysM-BDNF KO mice lacked innervation compared to the control group, which supports the emerging idea that adipose macrophages residing in the stromal vascular fraction, not mature adipocytes, secrete the neurotrophic factor, BDNF. Furthermore, these results suggest that BDNF is crucial in supporting the growth and viability of neurons in the tissue since those mice without BDNF secretion were unable to sustain the innervation in their adipose tissue.

Results from hematoxylin staining showed that there was browning present in the adipose tissue of KO mice after cold exposure, which was surprising since it was previously thought that innervation was needed to initiate the process of adipose browning. The results from this study suggest that innervation is not required for the process of 'browning' to occur; that is, new recruitable or beige adipocytes can form within a WAT depot without neuronal stimulation. However, immunostaining with UCP1 anitbody showed that the recruitable brown adipocytes that were formed in the LysM-BDNF KO mice were deficient in the protein UCP1, which is needed to actually

initiate non-shivering thermogenesis in functional BAT. These findings indicate that innervation and proper activation of the sympathetic nervous system is required for the activation of UCP1-mediated thermogenesis.

It was difficult to properly asses the relative sizes of the adipocytes in the LysM-BDNF KO and control mice because of complications with the computer imaging program compatibility, however there did not seem to be any difference in cell size between the KO mice, which had a higher amount of very concentrated recruitable BAT in their WAT depots, and control mice, which exhibited a less dense, more evenly distributed amount of browning. This could indicate that the recruitable BAT in LysM-BDNF KO mice was not properly activated and therefore unable turn on thermogenesis, which would have utilized a high number of fatty acids from the WAT surrounding the very concentrated BAT causing adipocyte size to shrink.

Since vasculature was present in all depots, it is reasonable to hypothesize that a circulatory factor may initiate the browning process, but is not sufficient to provide the activation needed to initiate thermogenesis. Existing evidence suggests that the mural cell compartment found in vasculature serves as a reservoir for adipocyte precursors which are committed, either prenatally or early in postnatal life (Tihai et al., 2013, Tang et al., 2008). Also, an increase of adipose vascularization by means of increase in vascular endothelial growth factor (VEGF) has been shown to increase the presence of browning in WAT (Elias et al., 2012). Consistent with these findings, it is possible that a circulatory factor present in adipose vasculature could activate the available progenitor cells to proliferate into mature adipocytes, and that in response to cold, the proliferation of so-called “recruitable” type adipocytes could be responsible for browning in WAT.

An interesting aside from the results is that the unilocular WAT cells in the control mice expressed UCP1, which could suggest that the WAT depot was undergoing transdifferentiation into multilocular BAT which contains UCP1 and can undergo non-shivering thermogenesis. Further studies with cell lineage tracking could further illuminate the process of browning in WAT.

To my knowledge, this is the first study to make this distinction between the mechanisms that initiate browning (possibly a circulatory factor) and those that activate UCP1-mediated thermogenesis (innervation). These findings create a new layer of complexity in the attempt to understand adipose biology. Further studies will need to be done to confidently determine what is controlling the initiation of the browning process if it is not innervation and how that factor works along side innervation in cold-induced browning of WAT and thermogenesis.

In conclusion, this study demonstrates innervation in adipose tissue is dependent on LysM cell-secreted BDNF and that innervation is needed to activate UCP1 mediated thermogenesis in the recruitable cells within a WAT tissue, although it is not necessary to initiate the process of WAT browning. Along with the previously understood importance of adipose tissue in metabolic health, these findings help to increase the understanding of the adipose browning mechanism and could be instrumental in developing future therapeutic options for those battling metabolic disease

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Appendix A: Microscope Protocol

How to Analyze Adipose Histology on the Microscope,
Townsend Lab as of September 2015

1. Organization of data: Project → Cohort → Animal # → Slide # → Photos
2. For each Cohort, the blocks of tissues are typically sliced on the microtome so we have 6 slides of 2-3 tissue sections per slide. Once all animals in a cohort are sliced for a given tissue (ie: scWAT), then we can move onto histology. Only stain when you have a full cohort to stain all together.
3. Typically, the 6 slides per animal tissue, we use slide #3 for Hematoxylin stain. This is the slide you will be analyzing on the microscope. This stain allows us to see the morphology of the cells, so we can ask:
    a. What did the experiment do to the cell size?
    b. Is there Multiolocularity? Is there browning in WAT or did the BAT lipid droplet change size?
    c. Is there vascular input?
    d. Are there crown-like structures?
    e. Other observations?
4. Some of these will be simply qualitative observations, others can be quantified in Image J based on the photos taken on the scope.

For each slide:

1. Systematically visualize the entire tissue in a grid-like pattern, left to right and top to bottom.
2. As you go along, make notes on the template form to note your observation about the morphology. Take photos of anything noteworthy (browning, crown-like structures at 200x total magnification).
3. Once you have viewed the entire tissue section, go back and take 3 representative images that capture the average or overall look of the tissue. (Be diligent to avoid bias- best done by an observer who is blind to the experiment). Save images as TIFF files.
4. Save image files by animal #, slide # and photo info. The images on the camera will automatically save by data and time, you can annotate the file afterwards. Store these images in folders by Project/ Cohort / Experiment.

Author's Biography

Parents Larry Wood and Jane Fallgren barely made it to Fair Oaks Hospital in time for the birth of Elizabeth (Liz) Wood on December 28, 1993. She graduated from Liberty High School in Bealeton, VA in June of 2012 and continued to the University of Maine on a full basketball scholarship to pursue a major in biology with a pre-med concentration and a minor in chemistry while also being a member of the Honors College. She also serves on the Division I Student Athlete Advisory Committee at the campus, conference and national level.

She was named third team All-American and received the America East Conference Elite 18 award, the M Club's Dean Smith Award as well as the Frank B. and Charles S. Bickford Prize from the University of Maine College of Natural Sciences, Forestry and Agriculture.

Upon graduation, Liz plans to take a year before applying to medical school to play professional basketball overseas.