

ScholarWorks@GSU

Peripheral Sympathetic Leptin Receptor Signaling Regulates Sympathetic Innervation of White Adipose Tissue and Diet-Induced Thermogenesis

Authors	Silva, Felipe C
Citation	Silva, Felipe C. 2023. "Peripheral Sympathetic Leptin Receptor Signaling Regulates Sympathetic Innervation of White Adipose Tissue and Diet-Induced Thermogenesis." Georgia State University. https://doi.org/10.57709/36391854
DOI	https://doi.org/10.57709/36391854
Download date	2026-03-12 16:57:34
Link to Item	https://hdl.handle.net/20.500.14694/2083

Peripheral Sympathetic Leptin Receptor Signaling Regulates Sympathetic Innervation of White
Adipose Tissue and Diet-Induced Thermogenesis

by

Felipe Silva

Under the Direction of Bingzhong Xue, PhD

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

in the College of Arts and Sciences

Georgia State University

2023

ABSTRACT

The rising prevalence of obesity poses a significant public health challenge, with profound implications for metabolic disorders and associated health risks. In the United States, approximately 40% of adults are considered obese. Obesity is caused by a prolonged increase in energy intake coupled with a decrease in energy expenditure. Adipose tissue is a key player in the regulation of energy balance. There are three different types of adipocytes that compose the adipose tissue organ. White adipocytes are specialized for energy storage in the form of triglycerides while brown and beige adipocytes excel in energy dissipation via thermogenesis. Brown adipocytes are located in anatomically defined areas such as the intrascapular region (iBAT) and beige adipocytes are dispersed in clusters within larger white adipose tissue depots. Adipose tissue is densely innervated by the sympathetic nervous system (SNS), which is indispensable in the induction of adipocyte thermogenesis and lipolysis. The SNS responds to cold stimulus and excessive feeding by releasing catecholamines, which in turn activate brown/beige adipocyte thermogenesis and lipolysis. The activation of brown and beige adipocyte thermogenesis increases energy expenditure and promotes weight loss. Metabolic research has primarily focused on the investigation of neuroendocrine pathways regulating SNS activity. Hence, there exists a significant gap in knowledge regarding the role of SNS-targeted tissues, such as adipose tissue, in the regulation of SNS activation and development. The adipose tissue-derived hormone, leptin, has emerged not just as a master regulator of energy homeostasis, but also potent modulator of nervous system development. In this study, we utilize a knockout mouse model with sympathetic neuron specific deletion of the leptin receptor and additional transgenic mouse models to investigate the role of peripheral sympathetic leptin signaling in

whole-body energy homeostasis. We have discovered a novel mechanism by which sympathetic leptin signaling regulates SNS innervation of adipose tissue and diet-induced thermogenesis.

INDEX WORDS: Leptin, Leptin Receptor, Thermogenesis, Sympathetic Nervous System, Energy Homeostasis, Innervation

Copyright by
Felipe Carrasco Silva
2023

Peripheral Sympathetic Leptin Receptor Signaling Regulates Sympathetic Innervation of White
Adipose Tissue and Diet-Induced Thermogenesis

by

Felipe Silva

Committee Chair: Bingzhong Xue

Committee: Hang Shi

Vincent Rehder

Electronic Version Approved:

Office of Graduate Services

College of Arts and Sciences

Georgia State University

December 2023

DEDICATION

I dedicate this dissertation to Dr. Liana Artinian. Liana's guidance was indispensable during the early years of my PhD journey. She will be dearly missed

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my PhD advisor and mentor, Dr. Bingzhong Xue, for her support and guidance throughout my PhD journey. Dr. Xue's expertise, insightful perspectives, patience, and encouragement have been instrumental in my development as a scientist. Dr. Xue's support extends beyond the scope of my research, embracing not just my academic pursuits but also my personal goals and teaching endeavors. I truly cannot thank her enough.

I would next like to thank my committee members, Dr. Hang Shi and Dr. Vincent Rehder. Dr. Shi has had a pivotal impact in the development of my critical thinking skills and presentation skills. He is always happy to share his immense knowledge and provide insightful feedback during lab meetings and presentations. Dr. Vincent Rehder played an indispensable role in my becoming a scientist. After taking a neurobiology course with him as an undergraduate student, I was able to join his lab as an undergraduate researcher. I cannot thank him enough for providing me with this opportunity during a time in my life in which I was unsure of what to do next.

I would like to thank Dr. Stephanie Gutzler, who has been an incredible mentor for helping me develop my pedagogical skills and supporting my teaching aspiration. I would like to thank Dr. Jessica Carter, who I consider both a mentor and great friend. I thank all the faculty and staff at the Biology Department and animal facility staff for all their hard work.

I want to express my appreciation for all the friendships I've developed during my time at GSU. Especially Miranda Movahed and Shirong Wang. I thank all current and previous lab members who were always eager to help in any way imaginable.

Finally, I would like to extend my sincerest appreciation to my family for their unwavering support, encouragement, and understanding during my PhD journey. To my mother, Monica Silva, you have been my inspiration for as long as I can remember, as you succeed in everything that you do. You have shown me that I can accomplish anything through hard work and determination. I cannot thank you enough for all you've given up in support of my aspirations. To my father, Luis Silva, you have always inspired me to ask questions, and always to think for myself. From an early age, you have instilled a sense of curiosity in me, which is the backbone of scientific research. To this day, I am always excited to learn new things just so I can share them with you. To my sister, Caroline Silva, you have always kept me grounded with your honest feedback and support throughout the years. Although I feel guilty for not being as present in your life as I should have been, I vow to be more supportive and present in the years ahead.

Last, but certainly not least, I would like to express my deepest gratitude to the most special person in my life, my wife, Madeline Silva. I truly cannot thank you enough for all the sacrifices you've had to make during my PhD journey. I am so grateful for your patience in listening to 30-minute lectures where I detailed every aspect of a topic, just to convey my excitement about a single graph's worth of data. You have been there with me through the ups and the downs. You've not only supported me but provided me with purpose and a desire to succeed. I truly could not have accomplished as much as I have without you by my side.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS		V
LIST OF TABLES		X
LIST OF FIGURES		XI
1 INTRODUCTION		1
1.1 The Obesity Epidemic and Adipose Tissue Biology		1
1.2 Sympathetic Regulation of Adipose Tissue		3
<i>1.2.1 Adrenergic Signaling</i>		<i>6</i>
1.3 Sympathetic Innervation of Adipose Tissue		8
<i>1.3.1 Known Modulators of Adipose Tissue SNS Innervation</i>		<i>10</i>
1.4 Leptin Regulates Energy metabolism		14
<i>1.4.1 Leptin-Leptin Receptor Signaling</i>		<i>15</i>
1.5 Leptin Signals via Central Pathways		16
1.6 Leptin as a Neurotrophic Factor		20
<i>1.6.1 Leptin Regulates SNS Development</i>		<i>21</i>
2 DISSERTATION GOALS		22
3 METHODS		24
3.1 Animals		24
3.2 Metabolic Measurements		25
3.3 Cold Exposure		25

3.4	Quantitative RT-PCR	26
3.5	RNA-Sequencing Analysis.....	26
3.6	Immunoblotting.....	26
3.7	Immunohistochemistry	27
3.8	Adipose Tissue Whole-Mount Clearing and Immunostaining	28
3.9	Sympathetic Neuronal Culture and Neurite Growth Measurements	28
3.10	Electrophysiology	29
3.11	Statistical Analysis	29
4	RESULTS	31
4.1	Leptin Receptor is Expressed in Sympathetic Ganglia and Regulates Sympathetic Neuronal Growth	31
4.2	6-Week-Old Female Mice with Sympathetic Neuron Specific Deletion of LepR Exhibit Decreased Energy Expenditure.....	35
4.3	Female Mice with Sympathetic Neuron Specific Deletion of LepR are Prone to Diet-Induced Obesity	42
4.4	LepR Signaling Regulates Diet-Induced Thermogenesis but Not Cold-Induced Thermogenesis in Adult Mice	49
4.5	LepR Signaling Regulates Sympathetic Axon Growth and White Adipose Tissue Innervation	53
4.6	LepR Regulated Mitochondrial Oxidative Metabolism is Important for Sympathetic Neuronal Growth.....	55

5	DISCUSSION	59
6	CONCLUSION	65
7	REFERENCES.....	75

LIST OF TABLES

Supplemental Table 1: Antibodies used in immunoblotting.....	74
--	----

LIST OF FIGURES

Figure 1. LepR is expressed in sympathetic ganglia and LepR signaling promotes neurite growth. (A) Representative β III-tubulin Immunofluorescent images of sympathetic ganglia neurons isolated from 6-week-old LepR-Cre::Ai14 reporter mice. (B) Representative images of TH, Fast Blue (FB) and LepRb-TdTomato labeling in L1 sympathetic ganglia. (C) Serum Leptin protein level in WT mice at different length cold challenges (N=6 for all measurements). (D) Quantitative RT-PCR analysis of Leptin mRNA expression in iWAT (RT n = 6, 1D Cold n = 5, 7D Cold n = 6). (E) Quantitative RT-PCR analysis of Leptin mRNA expression in BAT (n = 6). (F) Representative β III-tubulin immunofluorescent images of sympathetic ganglia neurons treated with Leptin (100 ng/ml for 12 hours) or PBS from 6-week-old LepR-Cre::Ai14 reporter mice and quantitation of the number of neurites per neuron, total neurite length per neuron, maximal neurite length per neuron, and average neurite length per neurite per neuron (PBS n = 24, Leptin n = 28). All data are expressed as mean \pm SEM; * $p < 0.05$ 34

Figure 2. 6-week-old female mice with LepR deficiency in sympathetic neurons exhibit decreased energy expenditure and increased adiposity. (A) Body weight at the time of analysis (n = 6). (B) Tissue weights (n = 6). (C) H&E staining of iWAT and BAT (iWAT n = 4 for both groups, BAT n = 3 for both groups). (D) Oxygen consumption (n = 6 for both groups; Light phase represented by white background; Dark phase represented by grey background). (E) Energy Expenditure measured as heat production (n = 6 for both groups; Light phase represented by white background; Dark phase represented by grey background). (F) Food intake measurement of 6-week-old females, measurements

recorded during the animal's 6th week of life (n = 6 for both groups). All data are expressed as mean \pm SEM; * p < 0.05. 39

Figure 3. 6-week-old female mice with LepR deficiency in sympathetic neurons exhibit decreased thermogenic gene expression and TH protein levels in iWAT and BAT. (A) Quantitative RT-PCR analysis of thermogenic gene expression in iWAT (n = 5-6 per group). (B) TH protein content in iWAT (n = 6). (C) Quantitative RT-PCR analysis of thermogenic gene expression in BAT (n = 5-6 per group). (D) TH and UCP1 protein content in BAT (n = 6). (E) UCP1 staining in iWAT and BAT (iWAT n = 3, BAT n = 4). All data are expressed as mean \pm SEM; * p < 0.05..... 40

Figure 4. 6-week-old male mice with LepR deficiency in sympathetic neurons display decreased iWAT thermogenic gene expression with no associated metabolic phenotype. (A) Body weight at the time of analysis (n = 6). (B) Tissue weights (n = 6). (C) Oxygen consumption (n = 6 for both groups; Light phase represented by white background; Dark phase represented by grey background). (D) Energy Expenditure measured as heat production (n = 6 for both groups; Light phase represented by white background; Dark phase represented by grey background). (E) Food intake measurement of 6-week-old males, measurements recorded during the animal's 6th week of life (n = 6 for both groups). (F) Quantitative RT-PCR analysis of thermogenic gene expression in iWAT (n = 5-6 per group). (G) Quantitative RT-PCR analysis of thermogenic gene expression in BAT (n = 6 per group). (H) UCP1 staining of iWAT (n = 2 for both groups). All data are expressed as mean \pm SEM; * p < 0.05. 41

Figure 5. Female mice with sympathetic neuron specific LepR deletion are prone to diet-induced obesity due to decreased energy expenditure. Female SLRKO and fl/fl littermates were

placed on HFD for 30 weeks when they were 6 weeks of age. (A) Body weight curve (n = 12 for both groups). (B) Body composition (fat mass n = 11 fl/fl, n = 12 SLRKO; lean mass n = 11 fl/fl, n = 12 SLRKO). (C) Tissue weight (n = 10 for both groups). (D) H&E staining for iWAT and BAT (WAT & BAT n = 3 for both groups). (E) Oxygen consumption (n = 12 for both groups; Light phase represented by white background; Dark phase represented by grey background). (F) Energy Expenditure measured as heat production (n = 12 for both groups; Light phase represented by white background; Dark phase represented by grey background). (G) Food intake measurement of females during week 24 on HFD, measurements recorded during the animal's 30th week of life (n = 4 for both groups). All data are expressed as mean \pm SEM; * p < 0.05. 45

Figure 6. HFD-fed female mice with sympathetic neuron specific LepR deletion exhibit decreased expression of thermogenic genes and TH protein content in iWAT. (A) Quantitative RT-PCR analysis of thermogenic gene expression in iWAT (n = 7-8 per group). (B) TH protein content in iWAT (fl/fl n = 7, SLRKO n = 8). (C) Quantitative RT-PCR analysis of thermogenic gene expression in BAT (n = 7-8 per group). (D) TH and UCP1 protein content in BAT (n = 7). All data are expressed as mean \pm SEM; * p < 0.05. 46

Figure 7. Male mice with sympathetic neuron specific LepR deletion exhibit decreased energy expenditure and a decrease in BAT weight. Male SLRKO and fl/fl littermates were placed on HFD for 30 weeks when they were 6 weeks of age. (A) Body weight curve (fl/fl n = 10, SLRKO n = 12). (B) Body composition (fl/fl n = 10, SLRKO n = 12). (C) Tissue weight (fl/fl n = 10, SLRKO n = 12). (D) H&E staining for iWAT and BAT (WAT & BAT n = 3 for both groups). (E) Oxygen consumption (fl/fl n = 10, SLRKO n =

12; Light phase represented by white background; Dark phase represented by grey background). (F) Energy Expenditure measured as heat production (fl/fl n = 10, SLRKO n = 12; Light phase represented by white background; Dark phase represented by grey background). All data are expressed as mean \pm SEM; * p < 0.05. 47

Figure 8. HFD-fed male mice with sympathetic neuron specific LepR deletion exhibit no changes in thermogenic gene expression. (A) Quantitative RT-PCR analysis of thermogenic gene expression in iWAT (n = 7-8 per group). (B) TH protein content in iWAT (fl/fl n = 7, SLRKO n = 7). (C) Quantitative RT-PCR analysis of thermogenic gene expression in BAT (n = 7-8 per group). (D) TH and UCP1 protein content in BAT (n = 8). All data are expressed as mean \pm SEM; * p < 0.05..... 48

Figure 9. HFD-fed female mice with sympathetic neuron specific LepR deletion exhibit impaired diet-induced thermogenesis. HFD-fed female SLRKO and fl/fl littermates were placed in thermoneutral conditions (30 °C) then fasted for 16 hours (represented by the gold background) and re-fed for 24 hours (light phase represented by white background; dark phase represented by the grey background). (A) oxygen consumption (n = 6-7). (B) Energy expenditure measured as heat production (n = 6-7). (C) Within groups mixed model ANOVA comparison of oxygen consumption (n = 6-7, multiple comparison calculated with Tukey HSD) . (D) Within groups mixed model ANOVA comparison of energy expenditure (n = 6-7, multiple comparison calculated with Tukey HSD). All data are expressed as mean \pm SEM; * p < 0.05. 52

Figure 10. Sympathetic LepR regulates iWAT sympathetic innervation and is required for the neurotrophic effects of Leptin. (A) Representative β III-tubulin Immunofluorescent images of sympathetic ganglia neurons isolated from 6-week-old SLRKO and fl/fl

female mice treated with phosphate-buffered saline (PBS) or Leptin (100 ng/ml) and quantitation of total neurite length per neuron (fl/fl + PBS n = 20, fl/fl + Leptin n = 16, SLRKO + PBS n = 16, SLRKO + Leptin n = 18). Statistical significance was analyzed with two-way ANOVA (* Indicates statistical significance with Tukey's multiple comparisons test). (B) Representative images of TH-stained nerve fibers in WAT of 6-week-old chow-fed SLRKO and fl/fl littermates. All data are expressed as mean \pm SEM; * p < 0.05. 54

Figure 11. RNA analysis of sympathetic ganglia from 6-week-old SLRKO and fl/fl females reveals defects in mitochondrial oxidative metabolism. L1 sympathetic ganglia RNA from SLRKO (n = 8) and fl/fl (n = 7) mice were diluted to equal concentrations and pooled into 2 samples, respectively. (A) GO pathway analysis of downregulated genes in SLRKO vs. fl/fl female mice. (B) Heatmap of the downregulated genes in SLRKO female mice compared to fl/fl littermates. (C) Volcano plot of differentially expressed genes involved in mitochondrial oxidative metabolism, contractile fiber function, and synaptogenesis (Log₂ fold change \geq 0.5) 58

Figure 12. Schematic illustration of the role of adipose tissue-derived leptin and LepRb signaling in the regulation of SNS innervation of adipose tissue. Leptin is secreted from adipose tissue acts through its receptor, LepRb, in sympathetic neurons to promote SNS innervation of iWAT and potentially iBAT..... 66

1 INTRODUCTION

1.1 The Obesity Epidemic and Adipose Tissue Biology

The prevalence of obesity has increased drastically over the past few decades (Flegal et al., 2016). Over 2 billion people, or around 30% of the global population, are currently classified as either overweight or obese (Caballero, 2019). In the United States, 41.9% of adults aged 20 or older are considered obese ($BMI \geq 30 \text{ Kg/m}^2$) (Stierman et al., 2021). This substantial surge in obesity rates is due to a growing prevalence of sedentary lifestyles coupled with the widespread availability of calorie-dense foods. More plainly, obesity is caused by a chronic energy imbalance resulting from an increase in energy intake coupled with a decrease in energy expenditure (Hill et al., 2012).

Obesity is associated with several secondary metabolic diseases such as type 2 diabetes, dyslipidemia, cardiovascular diseases, and various types of cancer (Caballero, 2019). Adipose tissue is a complex organ that plays an indispensable role in the regulation of whole-body energy homeostasis. There are two distinct types of adipose tissue in mammals, white adipose tissue (WAT) and brown adipose tissue (BAT). WAT comprises the largest adipose tissue volume in mammals and is critical for energy storage in the form of triglycerides. In contrast, BAT dissipates energy in the form of heat via adaptive thermogenesis (Kajimura et al., 2010).

The thermogenic activity of BAT is largely dependent on uncoupling protein 1 (UCP1), which uncouples oxidative phosphorylation from adenosine triphosphate (ATP) synthesis (Fedorenko et al., 2012). This results in a profound increase in energy expenditure as energy is dissipated as heat instead of being trapped in ATP. Although BAT adaptive thermogenesis was initially believed to only be active in smaller mammals and infant humans (Rosell et al., 2014;

Rosen & Spiegelman, 2000), recent studies have shown that brown fat is both present and active in adult humans (Cypess, 2009; Virtanen et al., 2009; van den Berg et al., 2017). When stimulated, BAT consumes more glucose per gram than any other peripheral tissue (Orava et al., 2011). Hence, understanding the underlying mechanism regulating BAT thermogenesis is paramount to combating the growing obesity epidemic. A third adipocyte type, the beige adipocyte, has been shown to express high levels of UCP1 and is therefore capable of performing adaptive thermogenesis. Furthermore, recent studies have proposed new UCP1-independent mechanisms for beige adipocyte thermogenesis including creatine substrate cycling (Kazak et al., 2015; Bertholet et al., 2017), and ATP-dependent Ca^{2+} cycling via SERCA2b (Atp2a2) and the Ca^{2+} release channel ryanodine receptor 2 (RyR2) (Ikeda et al., 2017).

Unlike traditional brown adipocytes that develop prenatally in anatomically defined areas, beige adipocytes are dispersed within WAT (Fedorenko et al., 2012). Indeed, beige adipocyte differentiation from both preadipocytes (Wu et al., 2012) and transdifferentiation from existing white adipocytes (Barbatelli et al., 2010) is induced by β -adrenergic stimulation and cold (Wang & Seale, 2016). In fact, the thermogenic capacity of both brown and beige adipocytes is highly dependent on direct β -adrenergic stimulation by the sympathetic nervous system (SNS) (Cao et al., 2019). Recent studies have shown that SNS innervation of WAT is required for beige adipocyte differentiation and thermogenesis (Cao et al., 2019).

The manipulation of adipose tissue in hopes of increasing brown and beige adipocyte numbers as well as overall thermogenic capacity is a logical therapeutic approach. Although cold exposure has been shown to be the most effective way to promote recruitment and activation of thermogenic adipose tissue, it is unrealistic to expect humans to subject themselves to cold environments for extended periods of time. Additionally, chronic cold exposure has been shown

to increase blood pressure and the risk of developing atherosclerosis (Dong et al., 2013). Hence, a better understanding of SNS induced thermogenesis is critical for combatting the current obesity epidemic.

1.2 Sympathetic Regulation of Adipose Tissue

It is currently well accepted that adipose tissue is under neuronal control from the sympathetic nervous system. Adipose tissue is directly innervated by efferent projections of SNS postganglionic neurons from the sympathetic chain. Sympathetic release of norepinephrine (NE) is critically involved in several adipose tissue metabolic processes such as lipolysis, thermogenesis, adipogenesis, and beiging (Hüicking et al., 2003; Koivisto et al., 1975). Furthermore, surgical or chemical SNS denervation results in the inhibition of lipolysis and thermogenesis in WAT and BAT (Cantu & Goodman., 1967; Cao et al., 2019; Demas & Bartness., 2001; Dullo & Miller., 1984).

Sympathetic outflow to adipose tissue is the primary initiator of lipolysis (Bartness et al., 2010; Youngstrom & Bartness., 1995). Studies over half a century ago hinted at SNS-driven lipolysis by electrically stimulating sympathetic fibers in WAT explants, which resulted in a rapid release of free fatty acids (FFAs) and glycerol (Correl, 1963). More recently, several studies have demonstrated that adipose tissue lipolysis is initiated as NE from SNS postganglionic neurons acts on adipocyte β -adrenergic receptors (β AR) to activate lipolytic enzymes that hydrolyze triacylglycerols (TAGs) into FFAs and glycerol (Bartness & Song., 2007). Furthermore, SNS regulation of lipolysis was elegantly supported via direct optogenetic stimulation of sympathetic nerve fibers in adipose tissue, which is sufficient to elicit a local lipolytic response (Zeng et al., 2015). In addition to NE, neuropeptide Y (NPY) is also released

by sympathetic nerve terminals and has been shown to inhibit lipolysis via signaling through its receptor NPYR1 (Bradley et al., 2005; Serradeil-Le Gal et al., 2000; Lundberg et al., 1990). Still, additional studies are needed to fully elucidate the role of NPY in the regulation of lipolysis. Taken together, the current studies hint at the complexity of SNS-induced lipolysis. Although WAT is the primary lipolytic adipose tissue, BAT and beige adipocytes also undergo lipolysis to generate FFAs as substrate for β oxidation, which powers UCP1-dependent thermogenesis (Cannon & Nedergaard, 2004). Additionally, BAT and beige adipocytes uptake FFAs in circulation to support UCP1-dependent thermogenesis (Shin et al., 2017). Due to the ability of BAT and beige adipocytes to actively oxidize lipids or use FFAs present in circulation, they are an excellent therapeutic target for decreasing fat mass.

The SNS has long been implicated in the regulation of body temperature (Maickel et al., 1967). Since then, sympathetic innervation has emerged as a critical regulator of BAT thermogenesis (Martinez-Sanchez et al., 2022). Indeed, surgical and pharmacological denervation of BAT results in a decrease in thermogenic capacity in addition to an increase in fat mass without affecting food intake (Bartness & Wade, 1984; Cao et al., 2019; Labbé et al., 2018; Takahashi et al., 1992). More recently, Lyons et al. (2021) optogenetically stimulated sympathetic nerve fibers within BAT and observed an increase in UCP1 expression coupled with an increase in core temperature in mice (Lyons et al., 2020). Unfortunately, the authors did not report any changes to body weight, fat mass, or whole-body energy expenditure. Therefore, evidence supporting the sufficiency of BAT activation in regulating body weight and energy expenditure is still lacking.

SNS signaling induces the differentiation and activation of beige adipocytes in clusters within WAT (Martinez-Sanchez et al., 2022). The process of beige adipocyte differentiation

from pre-adipocytes or transdifferentiation from existing white adipocytes is termed browning or beiging. The beiging process has been shown to occur in response to cold exposure in inguinal WAT (iWAT) areas containing the highest density of sympathetic nerves (Chi et al., 2018). Additionally, the reduction of neonatal beige adipocytes starting at postnatal day (P) 20 (Xue et al., 2007) temporally coincides with a decrease in Tyrosine Hydroxylase (a marker of sympathetic nerve fibers) protein expression in WAT (Cui et al., 2021). Subsequent studies performed by Dr. B. Xue's research group have shown that this neonatal beiging occurs at least partially via leptin-induced sympathetic innervation of iWAT (Wu et al., 2020). In accordance, SNS denervation of iWAT resulted in a complete ablation of cold-induced beiging (Cao et al., 2019).

In addition to its role as principal regulator of adipose tissue thermogenesis and lipolysis, the SNS also regulates adipogenesis (Puente-Ruiz & Jais, 2022). Specifically, SNS-derived NE inhibits adipogenesis (Jones et al., 1992). Indeed, SNS denervation of WAT increases preadipocyte differentiation into mature white adipocytes (Bowers et al., 2004; Foster & Bartness, 2006). Overall, most studies exploring mouse models with decreased adipose tissue innervation also report either an increase in adipocyte quantity (Hyperplasia) or adipocyte size (hypertrophy) (Chi et al., 2018; Cui et al., 2021; Wang et al., 2020).

1.2.1 Adrenergic Signaling

Sympathetic signaling occurs via norepinephrine and three isoforms of β -adrenergic receptors (β -AR), β 1-AR, β 2-AR and β 3-AR, the latter being the most abundantly expressed in murine adipose tissue (Granneman et al., 1991). Notably, genetic deletion of all three β -ARs (β -less) results in mice that are obese and intolerant to cold (Bachman et al., 2002). Additional studies targeting specific deletions of each individual β -AR isoform have elucidated their individual roles in the aforementioned β -less metabolic phenotype. For instance, although β 1-AR KO mice exhibit a normal basal metabolic rate, they are mildly deficient in cold-induced and diet-induced thermogenesis (Ueta et al., 2012). In contrast, β 2-AR KO mice exhibit normal thermogenic capacity and body weight coupled with a mild disruption in glucose homeostasis (Fernandez et al., 2014). Initial phenotypic characterizations of β 3-AR KO mice described only mild differences in thermogenic capacity and fat accumulation, even in response to HFD feeding (Susulic et al., 1995). More recent studies, however, reported that β 3-AR KO mice exhibit a significant increase in body weight and fat mass compared to littermate controls on HFD (Preite et al., 2016), and impairment in cold-induced thermogenesis and reduction in white adipocyte beiging (Barbatelli et al., 2010). This discrepancy between early and more recent studies could be due to a compensatory increase of β 1-AR in cold acclimated β 3-AR KO mice (Mattsson et al., 2011) and length of HFD feeding. Regardless of the initially observed mild phenotype of β 3-AR KO mice, signaling via β 3-AR has been a primary focus of metabolic research.

Functionally, NE binding to β 3-AR results in the activation of adenylyl cyclase, which converts ATP into cyclic AMP (cAMP). As a second messenger, cAMP binds to the inhibitory regulatory subunit of protein kinase A (PKA) resulting in the dissociation of the inhibitory

subunit and subsequent activation of PKA (Sчена & Caplan, 2019). Activated PKA can phosphorylate transcriptional factors such as cAMP response element-binding protein (CREB), peroxisome proliferator-activated receptor (PPAR) γ , and peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α) (Delghandi et al., 2005). Overall, NE- β 3-AR signaling results in increased expression and activation of several lipolytic markers such as Hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) (Christoffolete et al., 2004) as well as thermogenic markers such as UCP1 (Delghandi et al., 2005) and iodothyronine deiodinase 2 (DIO2) (Christoffolete et al., 2004).

Functional evidence for NE- β 3-AR signaling in adipose tissue comes from several studies employing the use of the selective β 3-AR agonist CL-316,243 (CL). Indeed, CL administration directly into WAT and BAT results in a robust increase in thermogenesis, fatty acid oxidation, and a decrease in food intake (Albert et al., 2016; Grujic et al., 1997; Himms-Hagen et al., 1994; Mottillo et al., 2014; Susulic et al., 1995). Despite these well accepted findings, the KO of β 3-AR in BAT does not impair adaptive thermogenesis due to a cold challenge (Mattsson et al., 2011; Susulic et al., 1995). In contrast, β 3-AR deficiency in WAT results in a decrease in thermogenic capacity (Jimenez et al., 2003). Furthermore, BAT specific re-expression of β 3-AR in otherwise β 3-AR null mice does not reinstate the previously observed effect of CL on thermogenesis. However, re-expression in both BAT and WAT fully reinstates the effect of CL on thermogenesis (Grujic et al., 1997). Taken together, this suggests that β 3-AR signaling in BAT is neither necessary nor sufficient to induce thermogenesis in mice.

Moreover, genetic impairment of lipolysis by deleting adipocyte triglyceride lipase (ATGL) in BAT did not impair β 3-AR and cold-induced thermogenesis, but deletion in both BAT and WAT did (Schreiber et al., 2017). This was a surprising result since lipolysis is a

critical source of FFAs to fuel thermogenesis. However, β 3-AR stimulation and cold exposure increase BAT uptake of FFAs from circulation (Bartlett et al., 2011; Wu et al., 2006). The source of the FFAs may be cardiac muscles (Schreiber et al., 2017) or WAT (Shin et al., 2017). Overall, these findings suggest that β -AR signaling and therefore sympathetic input to both WAT and BAT are necessary for thermoregulation and whole-body energy metabolism.

1.3 Sympathetic Innervation of Adipose Tissue

Although catecholaminergic signaling has long been known to cause WAT lipolysis, the source of catecholamines was largely believed to originate from the adrenal medulla (Bartness et al., 2014). This original assertion was likely due to the fact that studying sympathetic innervation of WAT had proven very difficult with conventional histological techniques. Due to the relative sparse innervation of adipose tissue, especially WAT, structural analysis of nerve fibers is difficult in sectioned samples. However, with recent advances in light-sheet microscopy and tissue clearing techniques, it is now possible to completely visualize adipose tissue innervation (Chi et al., 2018). Recent studies using catecholaminergic reporter animals in conjunction with synaptophysin labeling revealed that the overwhelming majority of adipose tissue innervation is indeed sympathetic (Zeng et al., 2015; Jiang et al., 2017).

Retrograde tracing techniques were also employed to map the central origins of SNS outflow onto adipose tissue. Early fluorogold retrograde tracing studies in Siberian hamsters showed that sympathetic nerves innervating iWAT were projected from cell bodies present in T13 sympathetic ganglion (Youngstorm & Bartness, 1995). Further use of polysynaptic tracers such as pseudo rabies virus (PRV), has allowed for the identification of several brain areas that project to both WAT and BAT. Commonly labeled hypothalamic areas are the preoptic area

(POA), dorsomedial hypothalamus (DMH), and paraventricular nucleus (PVH) (Foster et al., 2010; Yu et al., 2018; Nguyen et al., 2017). Indeed, lesions in the PVH result in an increase of fat accumulation (Foster et al., 2010), while norepinephrine injections into the DMH result in an increase of serum FFAs and a decrease in triacylglycerols (Zaia et al., 1997).

Recent studies employing the use of PRV injections into iWAT or BAT of sympathetic TH-tomato reporter animals coupled with whole torso clearing has allowed investigators to identify the preganglionic and postganglionic inputs to iWAT and BAT. These findings support previous observations that iWAT is primarily innervated by sympathetic neurons in the T13/L1 sympathetic ganglia and that these neurons are innervated by preganglionic inputs from the intermediolateral nucleus (IML) of T7-L2 in the spinal cord (Huesing et al., 2021). BAT on the other hand is directly innervated by postganglionic neurons in the T1-T5 sympathetic ganglia, which are in turn innervated by T1-T5 preganglionic neurons in the IML (Francois et al., 2019). Hypothalamic neurons can connect to cholinergic preganglionic neurons of the SNS directly and indirectly via connections to the hindbrain. Hypothalamic neurons that directly project to cholinergic preganglionic SNS neurons that are consistent with the spinal cord levels that innervate iWAT and BAT include, oxytocin and vasopressin-expressing neurons in the PVN (Bains & Ferguson, 1995; Badoer, 2001)), and pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) -expressing neurons in the lateral hypothalamus (LH) and arcuate of the hypothalamus (ARC) (Farzi et al., 2018; Elias et al., 1999). However, their role in adipose tissue regulation remains a mystery. Indirect connections to preganglionic SNS neurons occur via premotor neurons sites in the hindbrain. Commonly labeled hindbrain areas are the nucleus of the solitary tract (SOL), intermediate (IRt) and paraventricular reticular nuclei (PCRt) (Nakamura et al., 2017), and the raphe pallidus (RPa) (Nguyen et al., 2017).

Furthermore, multiple studies have demonstrated that RPa input to the SNS mediates BAT thermogenesis in response to a cold challenge (Dib et al., 1994; Kong et al., 2012).

1.3.1 Known Modulators of Adipose Tissue SNS Innervation

Sympathetic nervous system development and the subsequent innervation of adipose tissue is a complex process. Postganglionic sympathetic neurons originate from neural crest cells (NCCs) that migrate ventrally from the neural tube toward the dorsal aorta to form sympathetic ganglia along a sympathetic chain (Scott-Solomon et al., 2021). Upon completion of initial proliferation and differentiation, mature sympathetic neurons must then begin to develop axons and dendrites. The process in which the SNS establishes functional circuits with peripheral organs such as adipose tissue can be separated in four distinct growth stages: (1) Initiation of axon growth, (2) Proximal axon extension, (3) Distal axon extension and target innervation, (4) Neuron survival and growth inhibition signals (Scott-Solomon et al., 2021).

During early development, postganglionic sympathetic axons experience rapid elongation and extension alongside arteries as they are attracted by vascular-derived neurotrophins such as neurotrophin 3 (NT3) (Elshamy & Ernfors., 1996; Enomoto et al., 2001). Interestingly, Makita and colleagues demonstrate that postganglionic neurons of the superior cervical ganglia (SCG) actively distinguish and “choose” to project either along the internal or external carotid arteries leading to target innervation of the face or salivary glands, respectively. A subset of SCG neurons expressing the endothelin receptor type A (EdnrA) receptor for the neurotrophin endothelin-1, which is only produced by the external carotid, primarily innervates the salivary glands (Makita et al., 2008). These findings suggest that distinct neuron subtypes with varied

expression profiles exist prior to final target innervation. Furthermore, tissue specific signals expressed during early development may play a role in sympathetic axon elongation.

Sympathetic innervation of target tissues starts during embryonic stages and continues for several weeks into the postnatal period (Scott-Solomon et al., 2021). Sympathetic innervation of WAT can be observed as early as P6. Although at this stage, sympathetic fibers are still wrapped around major blood vessels with very few nerve endings visible in the adipose tissue parenchyma (Chi et al., 2021). The key signal controlling early stages of adipose tissue innervation is the neurotrophin nerve growth factor (NGF). Consistent with its role in early adipose tissue innervation, NGF is highly expressed in undifferentiated preadipocytes and decreases in mature white adipocytes (Kim et al., 2016; Peeraully et al., 2004). Furthermore, SNS specific deletion of the NGF receptor, Tropomyosin receptor kinase A (TrkA) results in a robust decrease in WAT innervation (Jiang et al., 2017).

Other neurotrophic factors from the same family as NGF, such as brain derived neurotrophic factor (BDNF) and NT3, have been shown to modulate adipose tissue SNS innervation. BDNF is of interest since it is an important modulator of whole-body energy homeostasis, through its actions in the CNS (An et al., 2015; Rios et al., 2001). Localized deletion of BDNF in the ventromedial hypothalamus results in the development of obesity due to an increase in food intake (Unger et al., 2007), while both central and peripheral administration of BDNF decreases food intake and increases energy expenditure (Nakagawa et al., 2000; Nonomura et al., 2001). Although the peripheral effects of BDNF have not been extensively interrogated, several studies have identified BDNF expression in adipose tissue (Bernhard et al., 2013; Hausman et al., 2006). Subsequent studies targeting adipocyte-specific deletion of BDNF and its receptor, Tropomyosin receptor kinase B (TrkB), did not result in metabolic phenotypes

or a decrease in adipose tissue BDNF levels (Nakagomi et al., 2015). These findings suggest that adipocytes are not the source of adipose tissue BDNF. A more recent study conducted by Blaszkiewics et al. (2020) elucidated the cellular source of adipose tissue BDNF and its effect on SNS innervation. BDNF appears to be synthesized by LysM⁺ myeloid cells in the stromal vascular fraction (SVF) of adipose tissue. Furthermore, LysM⁺ myeloid specific BDNF KO results in a decrease of both SNS innervation and thermogenic capacity of WAT (Blaszkiewicz et al., 2020). Although NT3's role in SNS development during the embryonic period has been known for decades, its specific role in promoting direct adipose tissue innervation has just recently been elucidated. Cui et al. (2021) has shown that NT3 is indeed expressed in adipose tissue and modulates SNS innervation of adipose tissue, through signaling via the Tropomyosin receptor kinase C (TrkC). Within BAT, NT3 expression is equally abundant in adipocytes as it is in the SVF. However, within WAT, NT3 expression occurred primarily in mature adipocytes. Furthermore, NT3 expression is maximized at P15-20, which temporally coincides with the appearance of developmentally induced beige adipocytes in WAT (Cui et al., 2021; Xue et al., 2007). An additional study by Bové et al. (2021) also underlines the effects of NT3-TrkC signaling on adipocyte differentiation, beiging, and overall thermogenic capacity. However, this study claims NT3 is primarily released by tissue-irrigating blood vessels instead of adipocytes within WAT (Bové et al., 2021). Therefore, further studies are needed to fully examine the cellular source of NT3 within adipose tissue.

Additional signals have been proposed to act as neurotrophic factors affecting SNS innervation of adipose tissue. These signals include Sarcolipin (Bal et al., 2017), PR domain containing protein 16 (PRDM16) (Chi et al., 2018), S100 calcium-binding protein (S100B) (Zeng et al., 2019), and Leptin (Wang et al., 2020). Leptin is of particular interest due to its well-

established effects on energy metabolism through discrete central pathways. Although leptin's role as a central neurotrophic factor has been observed for several years (Bouret et al., 2004; Bouret & Simerly., 2007; Komori et al., 2006), its effect on SNS innervation of adipose tissue remains understudied. Wang and colleagues (2020) show that leptin can act via central circuits in the arcuate nucleus (ARC) of the hypothalamus to regulate WAT and BAT innervation. Briefly, the authors claim that leptin-LepR signaling in POMC and agouti-related peptide (AGRP) neurons in the ARC regulate BDNF expression in neurons of the paraventricular nucleus (PVH). These BDNF-expressing PVH neurons in turn project directly to sympathetic preganglionic neurons in the spinal cord, providing a potential means for regulating postganglionic innervation of adipose tissue (Wang et al., 2020). Although, BDNF is known to act both retrogradely and anterogradely to promote axon growth and target tissue innervation (Altar et al., 1997; Choo et al., 2017; Fawcett et al., 1998; Wang et al., 2022), polysynaptic neurotrophic action such as the proposed PVH → SNS preganglionic neuron → SNS postganglionic neuron → adipose tissue by BDNF has not been previously described. Therefore, the proposed leptin-BDNF central pathway may involve additional unknown mechanisms at both the SNS preganglionic neuron → SNS postganglionic neuron and SNS postganglionic neuron → adipose tissue synapses. Moreover, the direct effects of leptin on SNS neuron growth and adipose tissue innervation remains unstudied.

1.4 Leptin Regulates Energy metabolism.

Leptin is a peptide hormone secreted primarily by adipocytes and has been shown to play an important role in central and peripheral regulation of energy homeostasis (Caron et al., 2018; Collins et al., 1996). Studies conducted in the 1950s by Ingalls and colleagues identified a recessive obese (*ob*) mutation in mice that as the name suggests resulted in the development of extreme obesity (Ingalls et al., 1950). However, it was not until 1994 that the *ob* gene was finally identified and shown to be primarily expressed in adipose tissue as a 14-kDa polypeptide that would later be named, leptin (Zhang et al., 1994). Initial studies showed that leptin administration decreased body weight and adipose tissue weight in wild-type and *ob/ob* mice, which are leptin deficient (Pellemounter et al., 1995; Campfield et al., 1995). Interestingly, the depletion of fat mass after leptin treatment is distinct from what is observed after food restriction in a number of respects: leptin treatment mostly spares lean body mass and potentially stimulates glucose metabolism, while starvation results in a loss of lean body mass and causes insulin resistance (Duska et al., 2005; Halaas et al., 1995). Unfortunately, obesity resulting from leptin deficiency only accounts for a small percentage of human obesity. Most obese patients have high levels of circulating leptin due to massive fat storage but exhibit impaired responsiveness to leptin signaling (leptin resistance) (Gruzdeva et al., 2019). Circulating leptin levels are directly proportional to fat mass. Hence, leptin administration is not a viable means for treating most forms of obesity.

Leptin is an anorexigenic hormone, thereby reducing food intake and increasing energy expenditure. The brain is responsible for maintaining body weight homeostasis via regulating feeding behavior and energy expenditure. A low energy state (e.g. starvation) is characterized by

a sharp decrease in circulating leptin levels resulting in food seeking behaviors and complementary neuroendocrine responses to help conserve energy (Friedman, 2019). Conversely, as energy storages increase, circulating leptin levels increase, which restricts food intake while increasing energy expenditure (Obradovic et al., 2021). Leptin has a total of six receptors isoforms that are encoded by diabetes (*db*) gene, but only the long form of the receptor (LepRb), which is expressed in several tissues including the brain, has been shown to exert metabolic actions (Dodd et al., 2014; Masuo et al., 2008; Villanueva & Myers, 2008). This is where leptin has been most extensively studied, specifically in the arcuate nucleus (ARC) of the hypothalamus (Villanueva & Myers, 2008), where it acts on both Agouti-related protein (AgRP) and pro-opiomelanocortin (POMC) neurons to decrease food intake (Côté et al., 2018).

1.4.1 Leptin-Leptin Receptor Signaling

The leptin receptor is a class 1 cytokine family receptor that exists as six alternatively spliced variants referred to as, LepRa, LepRb, LepRc, LepRd, LepRe, and LepRf (Löllmann et al., 1997; Tartaglia et al., 1995). While all LepR isoforms contain a Janus kinase (JAK)-binding domain, only the LepRb isoform also includes signal transducer and activator of transcription (STAT)- binding sites (Baumann et al., 1996). The binding of leptin to LepRb results in receptor dimerization, which activates JAK2 kinase. The activated JAK2 phosphorylates itself and three tyrosine residues (Y985, Y1077, Y1138) on the intracellular C-terminal of the receptor. These phosphotyrosines in turn serve as binding sites for signaling molecules. Although the binding of leptin to LepRb activates a number of signaling pathways, its actions on energy homeostasis are primarily mediated via the JAK2/STAT3 and insulin receptor substrate (IRS)/phosphatidylinositol 3 kinase (PI3K) pathways.

Upon initial phosphorylation of the tyrosine residues on the C-terminal of the LepRb by JAK2, STAT3 binds to Y1138 and is subsequently phosphorylated by JAK 2 kinase. STAT3 phosphorylation is critical for the effects of leptin on body weight and overall energy homeostasis. Once phosphorylated, STAT3 moves from the cytoplasm to the nucleus, where it binds to *agrp* and *pomc* promoters, decreasing AgRP and increasing POMC expressions. Indeed, STAT3 deletion in these neurons increases AgRP and decreases POMC expression, resulting in decreased energy expenditure, hyperphagia, and obesity (Gao et al., 2004). Additionally, once phosphorylated JAK2 then phosphorylates IRS, which in turn regulates PI3K activity. The PI3K pathway is primarily responsible for the effects of leptin on the membrane potential of POMC and AgRP/NPY neurons (Hill et al., 2008; Morrison et al., 2005). As expected, inhibition of PI3K in the brain prevents leptin induced anorexia (Niswender et al., 2001)

1.5 Leptin Signals via Central Pathways

The relatively broad central and peripheral expression of LepRb in mice suggests that the diverse effects of leptin signaling are mediated via discrete tissues and signaling pathways. Although LepRb is expressed in many brain regions, it is concentrated in the ARC of the hypothalamus, ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), and Lateral hypothalamus (LH). By far the most extensively studied leptin signaling pathway takes place within the ARC of the hypothalamus, where leptin exerts antagonistic effects on POMC and AGRP/NPY neurons. This brain region is located adjacent to the median eminence, an organ with porous blood-brain barrier (BBB), potentially explaining how leptin enters the CNS. Indeed, the majority of leptin's action on ARC neurons is mediated by direct effects on neuronal projections close to the median eminence (Langlet et al., 2013; Balland et al., 2014). Leptin can

also be detected within the cerebral spinal fluid (CSF) suggesting that it is able to cross the BBB. Studies conducted in 2013 and 2014 suggested that leptin was transported into the CSF via tanycytes (Langlet et al., 2013; Balland et al., 2014). However, more recent studies have been unable to detect Leptin receptor in tanycytes (Yoo et al., 2019), potentially indicating a leptin receptor-independent mechanism for leptin transport via tanycytes (Yoo et al., 2020).

Within the ARC, AGRP/NPY and POMC neurons display an antagonistic relationship while regulating food intake. AGRP/NPY neurons activation promotes feeding via the release of AGRP and NPY, while POMC neuron activation inhibits feeding via the release of α -melanocyte stimulating hormone (α -MSH) (Elias et al., 1999). Leptin signaling hyperpolarizes AGRP/NPY neurons by opening K_{ATP} and BK channels (Shanley et al., 2022; Spanwick et al., 1997). Conversely, Leptin directly activates POMC neurons via depolarization through the opening of transient receptor potential-canonical channels (TRPC) (Qiu et al., 2010) and indirectly by inhibiting AGRP/NPY neurons (Cowley et al., 2001). This is possible because AGRP/NPY neurons co-express GABA and synapse with local POMC neurons in the ARC (Rau & Hentgens, 2017). More recent studies employing the use of a CRISPR-Cas9 mediated deletion of *LepRb* in either AGRP/NPY or POMC neurons have revealed that the effects of leptin on food intake are primarily mediated via AGRP/NPY neurons rather than POMC neurons (Xu et al., 2018). Indeed, *LepRb* deletion in AGRP/NPY neurons resulted in a drastic increase in food intake and body weight, while *LepRb* deletion in POMC neurons resulted in a mild increase in body weight without an increase in food intake (Xu et al., 2018). This finding is indicative that leptin regulates body weight homeostasis via other means besides food intake.

Evidence suggesting that leptin regulates body weight homeostasis via additional mechanisms has existed for almost two decades. In 1996, Levin and colleagues conducted pair-

feeding experiments in leptin deficient *ob/ob* mice treated with exogenous leptin. They observed that leptin-treated *ob/ob* mice lost significantly more weight than untreated but pair-fed *ob/ob* mice. Interestingly, the untreated *ob/ob* animals lost significantly more lean mass than the treated group (Levin et al., 1996). These findings indicate that leptin promotes energy expenditure through increased fat utilization independently of feeding. In contrast to the well-defined mechanisms of leptin-induced regulation of feeding, information regarding the role of leptin on the energy expenditure is limited. The hypothalamus is still the primary area of focus regarding leptin-induced regulation of energy expenditure. This is likely due to it being the primary site mediating the appetite-suppressing effects of leptin and its role in the regulation of thermogenesis. Lesions in the DMH and VMH are associated with a decrease in core body temperature (Landry et al., 2011; Monda et al., 1997), while stimulation of the DMH and VMH resulted in an increase in core body temperature (Halvorson et al., 1990; Zaretskaia et al., 2002).

Leptin injections into the DMH increase core body temperature without affecting food intake (Rezai-Zadeh et al., 2014). Interestingly, a study by Dodd et al. (2014) describes a distinct population of prolactin releasing peptide (PrRP) neurons responsible for partially mediating the observed thermogenic effects of leptin injections into the DMH. Furthermore, the thermogenic effects of leptin in the DMH are mediated via the SNS (Enriori et al., 2011). Evidence indicating that leptin activates certain neurons in the DMH is relatively strong (Dodd et al., 2014; Simonds et al., 2014), but LepRb is expressed in both GABAergic and glutamatergic neuron populations (Han et al., 2023; Xu et al., 2013). This raises ambiguity concerning both the pathway and mechanism by which leptin regulates energy expenditure via the DMH.

The VMH has also been implicated in leptin-induced regulation of energy expenditure. Leptin infusion into the VMH increases plasma catecholamine levels, an outcome that was abolished in VMH-lesioned animals (Satoh et al., 1997; Satoh et al., 1999). Moreover, the deletion of LepR in the VMH leads to an increase in adiposity (Bingham et al., 2008). Interestingly, VMH leptin infusions cause an increase in glucose uptake by BAT, which is prevented by sympathetic denervation (Minokoshi et al., 1999). Recent studies have focused on distinct neuronal populations within the VMH such as steroidogenic factor 1 (SF-1) neurons. LepRb deletion in SF-1 neurons results in obesity without hyperphagia (Dhillon et al., 2006), and when fed a high fat diet (HFD), SF-1 KO mice display impaired thermogenic capacity (Kim et al., 2010). In addition, neuron specific KO of LIM domain only 4 protein (LMO4) in the VMH results in impaired thermogenesis and the development of obesity in mice. However, exogenous leptin administration partially ameliorated the phenotype by increasing BAT thermogenesis compared to litter-mate controls (Zhou et al., 2012).

Lastly, leptin has also been shown to regulate energy expenditure by targeting AGRP/NPY and POMC neurons in the ARC. Functional evidence comes from several studies measuring SNS activity and thermogenesis. In addition to its effects on feeding, LepRb deletion within the ARC decreases SNS activity and diet induced thermogenesis (Harlan et al., 2011). Leptin's effect on the SNS activity and thermogenesis appears to be mediated by its inhibition of AGRP/NPY, which results in a decrease in NPY release. NPY displays potent orexigenic effects, resulting in increased food intake and decreased thermogenesis in rodents (Paul et al., 2005; Székely et al., 2005). Indeed, NPY overexpression and infusion in the ARC decrease SNS activity and BAT thermogenesis in rodents (Lopez-Valpuesta et al., 1996; Shi et al., 2013). As expected, due to their antagonistic nature, LepRb deletion in POMC neurons resulted in the

opposite effect compared to AGRP/NPY neurons. Germline deletion of LepRb in POMC neuron results in reduced SNS activity after leptin infusion compared to littermate controls (Balthasar et al., 2004; Do Carmo et al., 2011). However, induced POMC specific deletion of LepRb in adult mice did not alter energy expenditure compared to littermate controls (Caron et al., 2018). Both AGRP/NPY and POMC neurons project to the paraventricular nucleus of the hypothalamus (PVH) and VMH to regulate SNS activity and thermogenesis. Functional evidence comes from NPY infusions into the PVH, resulting in increased SNS activity and BAT thermogenesis (Billington et al., 1994; Kotz et al., 2000). In addition to PVH infusion of melanotan II, a melanocortin-4 (MC4) receptor agonist (receptor target of α -MSH) increases SNS activity (Haynes et al., 1999). Overall, there are several parallel pathways that mediate the effects of leptin on energy expenditure. One commonality they all share is their reliance on the SNS to enact these effects on metabolic tissues such as adipose tissue. Yet, the potential direct actions of leptin on the SNS remains unclear.

1.6 Leptin as a Neurotrophic Factor

In addition to its metabolic effects, leptin also displays potent trophic effects on nervous system development. Early studies by Bereiter and Jeanrenaud (1979, 1980) described that the brains of *ob/ob* and *db/db* (mice lacking functional LepR) were structurally distinct compared to littermate controls (Bereiter & Jeanrenaud, 1979; 1980). Subsequent studies by Ahima et al. (1999) described that the same mutant animals had reduced brain weight and abnormal levels of several growth-associated proteins in the hypothalamus, neocortex and hippocampus (Ahima et al., 1999). Furthermore, leptin treatment during early life could restore normal brain weight and developmental patterns (Steppan & Swick., 1999). Leptin acts as a powerful neurotrophic signal

that promotes the formation of ARC projection into the PVH. Leptin stimulates axonal growth of ARC neurons *in vitro* (Bouret et al., 2004). Additionally, leptin deficient *ob/ob* display a 5-fold decrease in ARC axons projecting to the PVH (Bouret et al., 2004). Consistent with earlier findings, exogenous leptin treatment of *ob/ob* neonates restores normal innervation patterns, while leptin treatment in adult *ob/ob* mice at P80 does not (Bouret et al., 2004). Taken together, these findings suggest that leptin exerts its neurodevelopmental effects during a critical neonatal period. However, leptin still exerts neuroplastic effects in adult *ob/ob* mice, resulting in synaptic rearrangement of AgRP/NPY and POMC neurons within the ARC (Pinto et al., 2004).

1.6.1 Leptin Regulates SNS Development

A recent study using adipose tissue clearing methods revealed that *ob/ob*, *db/db*, and leptin resistant diet-induced obese (DIO) mice display significantly decreased sympathetic innervation of WAT and BAT. Moreover, chronic peripheral leptin treatment of *ob/ob* adult mice restored WAT and BAT innervation (Wang et al., 2020). This is especially interesting since leptin treatment in adult *ob/ob* animals fails to restore normal hypothalamic development in the ARC and PVH (Bouret et al., 2004). In accordance, leptin stimulates sympathetic axon growth *in vitro* via STAT3 phosphorylation (Pellegrino et al., 2014). Furthermore, LepR has been detected in postganglionic neurons of the SNS system (Czaja et al., 2002; Miller et al., 1999). These findings suggest that leptin may directly regulate SNS development and adipose tissue innervation via LepR signaling in sympathetic neurons. However, the physiological relevance of peripheral sympathetic leptin signaling in SNS development and energy metabolism remains unstudied.

2 DISSERTATION GOALS

This dissertation is dedicated to unraveling the functional and physiological relevance of peripheral sympathetic leptin signaling in the regulation of whole-body energy homeostasis. We hypothesize that peripheral sympathetic leptin signaling regulates energy expenditure by modulating sympathetic innervation of adipose tissue in response to developmental and environmental cues. The hypothesis was tested across three major experiments.

Experiment 1: We first examined whether catecholaminergic postganglionic sympathetic neurons that directly innervate adipose tissue express LepRb. To this end we generated a transgenic mouse model that expresses a fluorescent protein in LepRb⁺ cells. Additionally, we also investigated the electrophysiological and trophic effects of leptin on sympathetic neurons *in vitro*.

Experiment 2: We next investigated the role of peripheral sympathetic leptin signaling in regulating whole-body energy metabolism by generating a novel, sympathetic neuron specific LepR knockout mouse model. We employed various metabolic characterization tools to investigate the role of peripheral sympathetic leptin signaling in regulating various metabolic parameters such as food intake, energy expenditure, glucose homeostasis, and adiposity. Metabolic characterization was replicated in adult and 6-week-old male and female mice to examine the potentially age dependent and sexually dimorphic effects of sympathetic leptin signaling.

Experiment 3: Lastly, we explored the potential functional and genetic mechanisms underlying the effects of peripheral sympathetic leptin signaling on whole body energy

metabolism. This was accomplished via RNA-seq analysis and by examining sympathetic innervation of adipose tissue *in vivo*.

Collectively, this dissertation stands as a pivotal advancement in our understanding of the mechanisms by which leptin regulates whole-body energy homeostasis. This work provides complementary evidence to leptin's role in increasing energy expenditure while uncovering sympathetic neurons as a previously unidentified target for direct leptin signaling. Furthermore, this study elucidates a novel mechanism through which sympathetic leptin signaling regulates energy metabolism.

3 METHODS

3.1 Animals

Mice with sympathetic-specific deletion of LepR (SLRKO) were generated by crossing LepR-floxed mice (LepR^{fl/fl}) with TH-Cre mice. LepR^{fl/fl} mice were obtained from Jackson Laboratories, Stock #008327 (Cohen et al., 2001); TH-Cre mice were also obtained from Jackson Laboratories Stock #008601 (Savitt et al., 2005). Therefore, LepR^{fl/fl} mice were used as the control group and LepR^{fl/fl}::THCre mice were used as the experimental group.

Reporter mice that express a red fluorescent protein variant (TdTomato) under the control of LepR promoter (LepR-Cre::Ai14) were generated by crossing LepR-Cre mice (Jackson Laboratories # 008320) (Defalco et al., 2001) with mice expressing a LoxP-flanked STOP cassette followed by TdTomato gene inserted into the *Gt(ROSA)26Sor* locus (Jackson Laboratories # 007914) (Madisen et al., 2010)

For Fast Blue injection, LepR-Cre::Ai14 mice were anesthetized via isoflurane, and a dorsal or ventral 2 cm incision was made to expose iBAT or iWAT. The retrograde tracer Fast Blue (FB) (2%; Polysciences, PA) was injected with a microsyringe into 5–10 separate loci (1 μ l/locus) of each adipose tissue. Animals were given 10–14 days to recover and allow for retrograde Fast Blue transport.

All animal procedures were approved by the Institutional Animal Care and Use Committee of Georgia State University and were in compliance with the Public Health Service and the United States Department of Agriculture guidelines.

3.2 Metabolic Measurements

All mice were housed with a 12/12 h light–dark cycle in temperature- and humidity-controlled rooms with free access to water and food (ambient temperature: 20–22 °C, thermoneutral temperature: 30 °C; humidity: 30–70%). SLRKO and littermate controls were fed either regular chow diet (LabDiet 5001, LabDiet, St. Louis, MO, 13.5% calories from fat) or high fat diet (HFD) (Research Diets D12492, 60% calorie from fat) for up to 40 weeks. Mice were housed at either ambient temperature (20–22 °C) or thermoneutrality (30 °C) to avoid any nonshivering thermogenesis that may be induced by mild cold stress in mice housed under ambient room temperature (20–22 °C). Various metabolic measurements were recorded as follows: (1) Body weight was monitored weekly. (2) Food intake, energy expenditure, and activity levels were measured using PhenoMaster metabolic cage systems (TSE Systems, Chesterfield, MO). (3) Body composition was analyzed using a Minispec NMR body composition analyzer (Bruker BioSpin Corporation; Billerica, MA). (4) Insulin sensitivity was determined by glucose tolerance and insulin tolerance tests (GTT and ITT, respectively) as previously described (Cui et al., 2021). At the end of metabolic measurements, various tissues including WAT, iBAT, muscle, liver, and sympathetic ganglia were collected for further analysis of gene-expression, protein-expression, and immunohistochemistry.

3.3 Cold Exposure

SLRKO mice and their respective littermate controls were subjected to chronic 7- day cold challenge (4 °C). Wild type mice were subjected to acute 6- hour, 1-day, and chronic 7-day

cold challenges (4 °C). At the end of the experiments, various tissues were collected for further analysis of gene-expression, protein-expression, and immunohistochemistry.

3.4 Quantitative RT-PCR

Total RNA from various tissues were isolated using Tri Reagent kit (Molecular Research Center, Cincinnati, OH) as previously described (Wang et al., 2016). The expression of genes of interest was measured by a one-step quantitative RT-PCR with a TaqMan Universal PCR Master Mix kit (ThermoFisher Scientific, Waltham, MA) using an Applied Biosystems QuantStudio 3 real-time PCR system (ThermoFisher Scientific) as we previously described (Cui et al., 2021). The quantitation of gene expression was normalized by the housekeeping gene cyclophilin. All Taqman primers and probes used were purchased from Applied Biosystems (ThermoFisher Scientific) or commercially synthesized.

3.5 RNA-Sequencing Analysis

RNA-seq library preparation, sequencing, and basic bioinformatics data analysis from SG, WAT, iBAT, and muscle were performed by BGI Americas (Cambridge, MA). Equal amount of RNAs from 6 animals/groups were pooled and used for RNAseq analysis. Differentially expressed genes between groups were defined as Log₂ fold change ≥ 0.5 or ≤ -0.5 .

3.6 Immunoblotting

Protein expression in adipose tissue was assessed by immunoblotting as previously described (Cui et al., 2021). Briefly, Tissues were homogenized in a modified radioimmunoprecipitation assay (RIPA) lysis buffer supplemented with 1% protease inhibitor

mixture and 1% phosphatase inhibitor mixture (SigmaAldrich, St. Louis, MO) and tissue lysates were resolved by SDS-PAGE. Proteins on the gels were transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA), which were then blocked, washed, and incubated with various primary antibodies, followed by Alexa Fluor 680-conjugated secondary antibodies (Life Science Technologies). The blots were developed with a Li-COR Imager System (Li-COR Biosciences, Lincoln, NE). The antibodies were listed in Supplemental Table 1.

3.7 Immunohistochemistry

WAT or iBAT tissues were fixed in 10% neutral formalin and embedded in paraffin, which was further cut into 5 μ m sections. The sections were either processed for hematoxylin and eosin (H&E) staining or immuno-staining with UCP1 antibodies as we previously described (Cui et al., 2021). Briefly, paraffin-embedded sections of iBAT and iWAT tissues were incubated with anti-UCP1 antibodies (Abcam, Boston, MA) overnight at 4°C and then incubated with biotin-conjugated anti-rabbit secondary antibodies (Jackson ImmunoResearch, West Grove, PA) for 30 min at room temperature.

Sympathetic ganglia were sectioned and immunostained as previously described (Cui et al., 2021). Briefly, SG were carefully harvested and transferred to an 18% sucrose solution in 0.1 M PBS containing 0.1% sodium azide at 4°C. All ganglia were then sectioned longitudinally at 10- μ m-thick sections using a cryostat. They were directly mounted onto slides (Superfrost Plus; VWR International, West Chester, PA) in four series with every fifth section on the same slide. Sections were then incubated with chicken anti-Tyrosin hydroxylase (TH 1:1000, Abcam, ab76442) antibody for 48 hours. Sections were then incubated with Cy3-donkey anti-rabbit (1:500; Jackson Immunoresearch, West Grove, PA) or Alexa Fluor 488-Donkey Anti-Chicken

(1:500; Jackson ImmunoResearch, West Grove, PA) secondary antibodies for 2h (supplementary table 1). Sections coverslipped using ProLong Gold Antifade Reagent (Life Technologies, Grand Island, NY). Images were captured using an Olympus DP73 photomicroscope and CellSens software (version 1.6) (Olympus, Waltham, MA). The captured images were evaluated with the aid of Image J software (version 1.5.2). The neurons were considered positively labeled based on the fluorescent intensity, cell size, and shape.

3.8 Adipose Tissue Whole-Mount Clearing and Immunostaining

A whole-mount adipose tissue clearing with the Adipo-Clear approach was conducted to allow immunostaining, followed by three-dimensional visualization and quantitation of sympathetic nerve innervation by light sheet microscopy. Briefly, adipose tissue was processed with a series of steps including dehydration, delipidation, and permeabilization, which was further stained with primary anti-TH antibodies (AB152, 1:1000, Millipore) and secondary antibodies (CyTM3 AffiniPure Donkey Anti-Rabbit IgG (H + L), 1:2000, Jackson ImmunoResearch, 711-165-152) (Supplementary Table 1). Tissues were further cleared with dibenzyl ether (DBE) and imaged with Bruker Multi-View (MuVi) Selective-Plane Illumination Microscope (SPIM). Three-dimensional image reconstruction was performed using the Imaris Image Analysis Software (version 9.5.1).

3.9 Sympathetic Neuronal Culture and Neurite Growth Measurements

SG at thoracic T1–T4 levels from 6- to 8-week-old mice were dissected and digested with collagenase I followed by Trypsin digestion. Dispersed neurons were cultured in complete neurobasal media (Fisher 10888022) with 1X B-27 (Fisher 17504044) and 200 μ M l-glutamine

(Fisher 25030149) in poly-d-lysine and Laminin coated dishes. Cells were cultured for 6, 12 or 36 hours with Leptin (100 ng/ml) in phosphate-buffered saline (PBS) or PBS as a control. Cells were then immunostained with Alexa Fluor® 488-conjugated antibodies against neurite growth marker β III-tubulin (1:400, AB15708A4, Millipore) (Supplementary Table 1). Neurite growth was then quantitated by Image J (version 1.5.2) with Neuron J plugin (version 1.4.3)

3.10 Electrophysiology

For electrophysiological recordings, sympathetic neurons were extracted from LepR-Cre::Ai14, SLRKO, and respective fl/fl littermates and dissected and digested as described above. Dissociated neurons were plated into 35 mm plastic dishes coated with poly-D-lysine and used for recording 2-3 days after plating. TdTomato+ sympathetic neurons were visualized using a mCherry/RFP-filtered excitation and emission fluorescent light under an inverted microscope. Patch-clamp recordings were performed using a Multiclamp 700B amplifier, Digidata 1440A interphase, and pClamp 10 software (Molecular Devices, Union City, CA) under voltage-clamp and current clamp modes. The external solution (pH 7.3) consisted of 152 mM NaCl, 2.8 mM KCL, 10 mM HEPES, 2 mM CaCl₂, and 10 mM glucose. The intracellular solution (pH 7.3) consisted of 130 mM K-gluconate, 10 mM HELES, 0.6 mM EGTA, 0.3 mM K-ATP, 0.3 mM Na-GTP, and 10 mM phosphocreatine.

3.11 Statistical Analysis

Data was expressed as mean \pm SEM. All graphs were created with GraphPad Prism (v9.1.5, GraphPad Software Group, San Diego, CA). The statistical tests were performed using

SPSS software (version 16.0, SPSS Inc, Chicago, IL, USA). Experimental data was analyzed by unpaired Student's t-test, one-way Analysis of Variance (ANOVA) followed by Bonferroni post-hoc analysis where appropriate, or two-way repeated measures ANOVA followed by Tukey's HSD post-hoc analysis where appropriate. For all experiments, differences among groups were considered statistically significant at $P < 0.05$.

4 RESULTS

4.1 Leptin Receptor is Expressed in Sympathetic Ganglia and Regulates Sympathetic Neuronal Growth

The adipokine leptin regulates energy homeostasis by targeting the hypothalamus. Indeed, genetic ablation of LepRb within the hypothalamus results in animals that are morbidly obese (Harlam et al., 2011). Unsurprisingly, metabolic researchers have primarily focused on understanding the central effects of leptin signaling. However, LepRb is expressed in various peripheral targets (Fei et al., 1997). To investigate if LepRb is expressed in the sympathetic nervous system, we generated a reporter mouse model, which expresses the red fluorescent protein, TdTomato in LepRb + cells (LepR-Cre::Ai14). This was achieved by crossing Ai14 mice designed with a loxP-flanked STOP cassette preventing transcription of TdTomato (Madisen et al., 2010) with LepR-Cre mice where Cre-recombinase is inserted immediately 3' of the stop codon in the last exon of the *LepR* gene (Defalco et al., 2001). This design ensures that TdTomato expression will be localized in cells that express the long form of the leptin receptor (LepRb). Sympathetic ganglia from 6-week old LepR-Cre::Ai14 reporter mice were dissected, digested and cultured *in vitro*. We observed robust expression of LepRb in sympathetic neurons as well as in non-neuronal cells in sympathetic ganglia (Fig. 1A). To further study whether LepRb is expressed in catecholaminergic neurons that specifically innervate iWAT, we injected the retrograde fluorescent neuronal tracer Fast Blue (FB) into iWAT of LepR-Cre::Ai14 reporter mice. Following FB injection, animals were allowed 10-14 days to recover and allow for retrograde transport of FB. Sympathetic ganglia in the lumbar L1 level, which innervates iWAT (Huesing et al., 2021), were dissected, sectioned, and stained with the catecholaminergic neuron marker tyrosine hydroxylase (TH) (Fig. 1B). Consistent with our *in vitro* observations, we

observed widespread expression of LepRb within the L1 sympathetic ganglia. Interestingly, ~ 8 TH+ FB+ LepRb+ neurons were observed, indicating that LepRb is expressed in catecholaminergic neurons that specifically innervate iWAT (Fig. 1B). Furthermore, the widespread TdTomato fluorescence potentially indicates that LepRb is expressed in non-neuronal glial cells in L1 ganglia (Fig. 1A-B). Similar results were also observed in thoracic T2 ganglia (Supplementary Fig. 1A), which innervates iBAT (Francois et al., 2019).

Cold exposure is a strong activator of the SNS and has been shown to activate adaptive thermogenesis and beigeing in iBAT and WAT, respectively (Cao et al., 2019; Wang & Seale, 2016). Moreover, Leptin has been shown to elicit adaptive thermogenesis in adipose tissue (Enriori et al., 2011; Zhou et al., 2012). Indeed, an acute 1-day cold challenge significantly increased serum leptin levels in 6-week-old mice, while a longer 7-day cold challenge did not (Fig. 1C). Additionally, iWAT leptin mRNA levels increased 10-fold and 5-fold in response to a 1-day and 7-day cold challenge, respectively (Fig. 1D). In contrast, leptin mRNA levels in iBAT were decreased in response to both cold challenges (Fig. 1E). Taken together, these data suggest that leptin may regulate SNS activity and thermogenesis during energetic challenges, such as cold exposure.

To elucidate the direct effects of leptin on sympathetic neurons, we first tested leptin's electrophysiological effects on sympathetic neurons *in vitro*. We found that leptin (100 nM) treatment significantly decreased the firing frequency of sympathetic neurons (2 ± 0.4 Hz) compared to PBS treated controls (13 ± 1.3 Hz) (Supplementary Fig. 1B). Additionally, leptin treatment significantly hyperpolarized the membrane potential of sympathetic neurons by -5.2 ± 1.0 mV compared to PBS treated controls (Supplementary Fig. 1C). Since leptin has been shown to act as a neurotrophic factor that regulates SNS neuron growth and adipose tissue innervation

(Pellegrino et al., 2014; Wang et al., 2020), we then tested leptin's effect on sympathetic neurite growth in primarily cultured sympathetic neurons from LepR-Cre::Ai14 *in vitro*. We observed that 100 nM leptin treatment significantly stimulated sympathetic neurite growth in LepRb+ neurons, as described by the increased total neurite length per neuron, maximum neurite length per neuron, and mean neurite length per neuron (Fig. 1F). Interestingly, these differences decreased with treatment time, and were no longer significant after 36 hours (Supplementary Fig. 1D-1E). This may indicate that leptin is especially important during early neurite development, synaptogenesis, and potentially adipose tissue innervation. All in all, our data strongly suggests that leptin directly regulates sympathetic neuron membrane potential, spike activity and neurite growth.

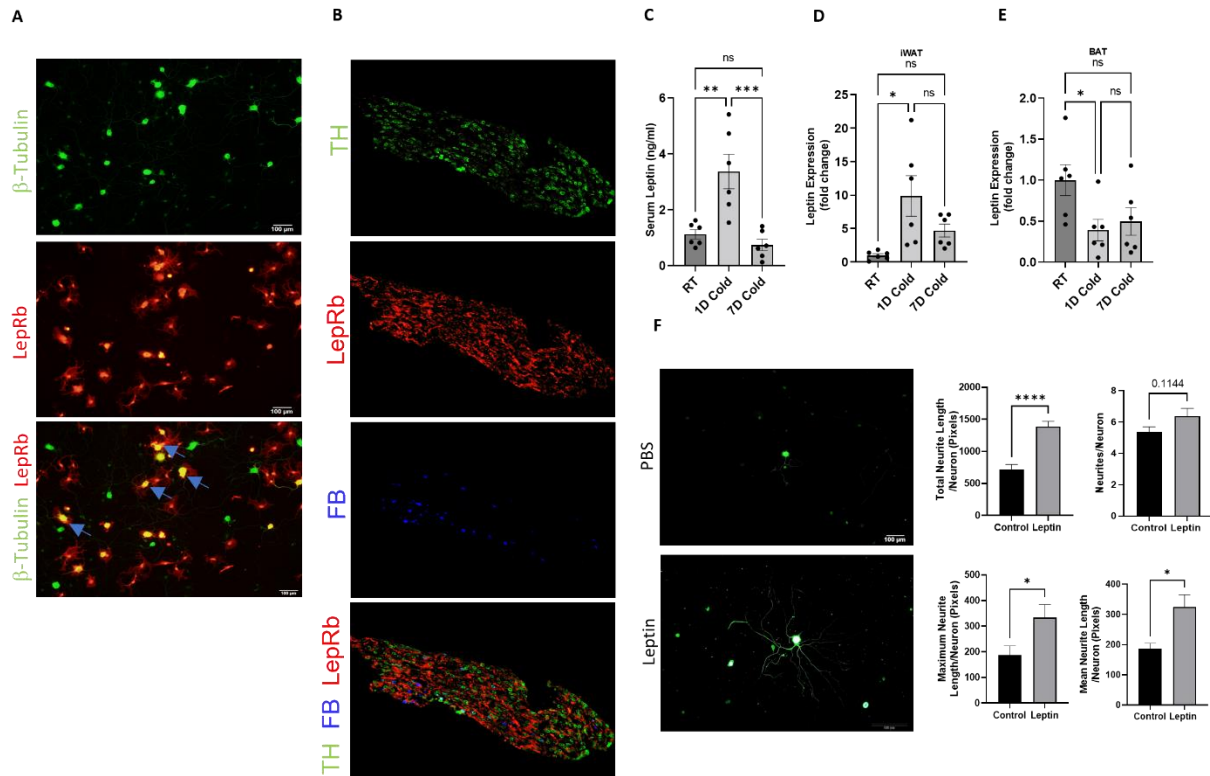


Figure 1. *LepR* is expressed in sympathetic ganglia and *LepR* signaling promotes neurite growth. (A) Representative β III-tubulin Immunofluorescent images of sympathetic ganglia neurons isolated from 6-week-old *LepR-Cre::Ai14* reporter mice. (B) Representative images of TH, Fast Blue (FB) and *LepRb-TdTomato* labeling in *L1* sympathetic ganglia. (C) Serum Leptin protein level in WT mice at different length cold challenges ($N=6$ for all measurements). (D) Quantitative RT-PCR analysis of Leptin mRNA expression in iWAT (RT $n = 6$, 1D Cold $n = 5$, 7D Cold $n = 6$). (E) Quantitative RT-PCR analysis of Leptin mRNA expression in BAT ($n = 6$). (F) Representative β III-tubulin immunofluorescent images of sympathetic ganglia neurons treated with Leptin (100 ng/ml for 12 hours) or PBS from 6-week-old *LepR-Cre::Ai14* reporter mice and quantitation of the number of neurites per neuron, total neurite length per neuron, maximal neurite length per neuron, and average neurite length per neurite per neuron (PBS $n = 24$, Leptin $n = 28$). All data are expressed as mean \pm SEM; * $p < 0.05$.

4.2 6-Week-Old Female Mice with Sympathetic Neuron Specific Deletion of LepR Exhibit Decreased Energy Expenditure

Leptin binds with high affinity to its cognate receptor, LepR. Leptin deficient *ob/ob* and LepR deficient *db/db* mice exhibit the same metabolic phenotype and decreased levels of SNS innervation of adipose tissue (Wang et al., 2020). LepR immunoreactivity has been detected in neurons within sympathetic ganglia of rodents (Miller et al., 1999) (Fig. 1B and Supplementary Fig. 1A), indicating the potential importance of leptin-LepRb signaling in regulating SNS-induced energy metabolism. Additionally, previous studies showed that leptin treatment in young *ob/ob* mice was able to restore normal hypothalamic innervation patterns, while leptin treatment in adult *ob/ob* mice did not (Bouret et al., 2004; Stepan & Swick., 1999). Therefore, we hypothesized that leptin-LepR signaling in sympathetic neurons may regulate SNS innervation of adipose tissue and whole-body energy metabolism in young, 6-week-old mice.

To explore the importance of leptin-LepR signaling in regulating axon growth, adipose tissue innervation and whole-body energy homeostasis, we generated mice with sympathetic-specific deletion of LepR. This was achieved by crossing LepR-floxed mice (fl/fl), where exon 1 of the *LepR* gene is flanked by loxP sites (Cohen et al., 2001) with TH-Cre mice where Cre-recombinase expression is under the control of the TH promoter (Savitt et al., 2005). As expected, LepR expression in sympathetic ganglia was reduced by ~ 40% in KO mice compared to fl/fl littermate controls, while no changes in LepR expression in the VTA (a brain area rich in catecholaminergic neurons) was observed (Supplementary Fig. 2A-2B and Supplementary Fig. 3A-3B). Furthermore, the ~ 40% decrease in LepR expression was measured via quantitative

RT-PCR analysis of the entire sympathetic ganglia, including non-neuronal cells. Since we observed LepRb expression in non-neuronal cells (Fig.1A-1B) and our animal model was designed to only delete LepR in sympathetic neurons, we expect the KO efficiency to be much higher among sympathetic neurons in the ganglia. Therefore, fl/fl mice were used as our control group and SLRKO mice were defined as being homozygous LepR-fl/fl and heterozygous TH-Cre (TH-Cre::LepR-fl/fl, or SLRKO). SLRKO mice were born with expected Mendelian frequency and displayed no noticeable developmental abnormalities.

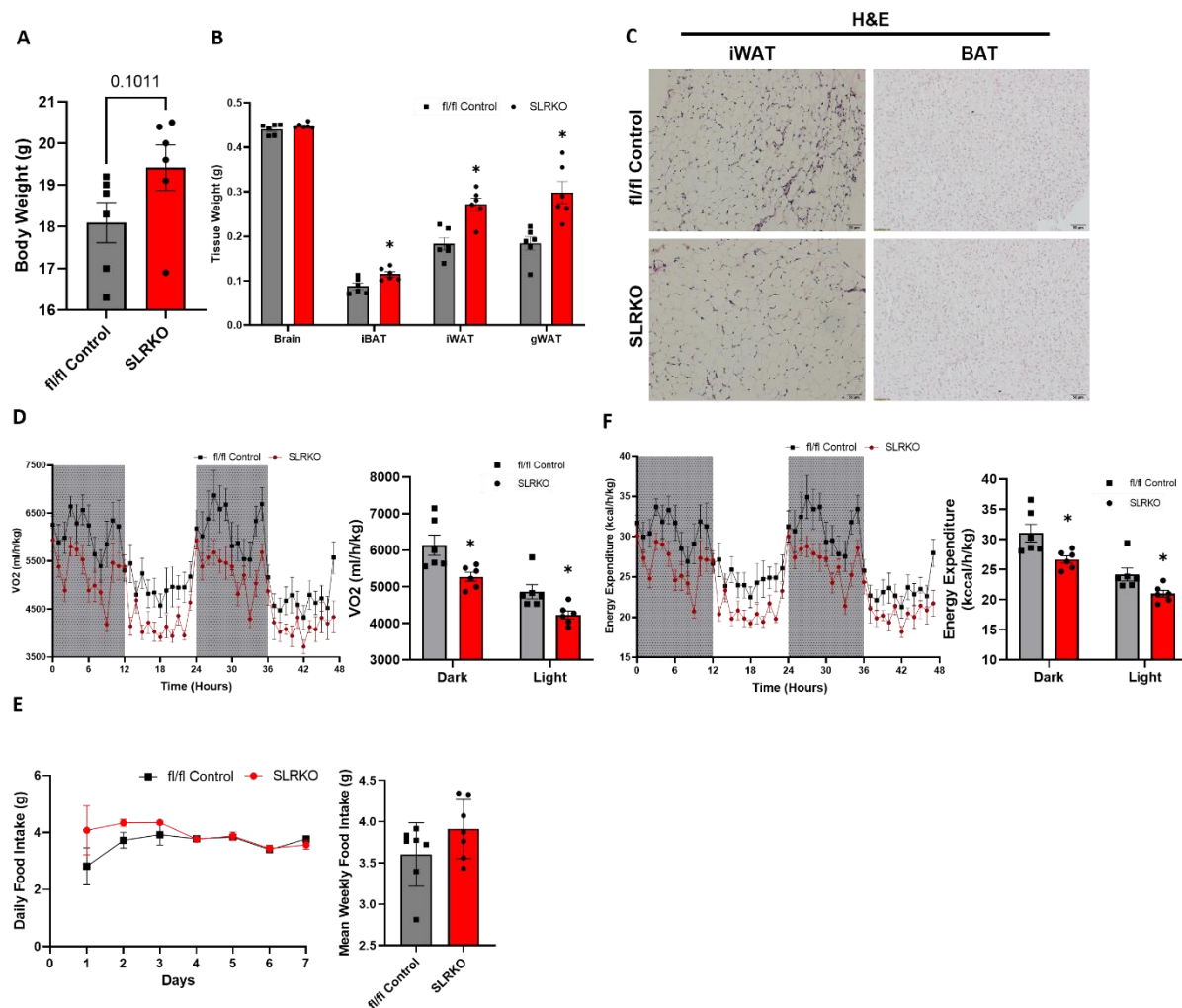
To elucidate the role of leptin-LepR signaling in young 6-week-old mice, we conducted metabolic characterization of 6-week-old SLRKO and fl/fl control mice fed standard chow diet *ad libitum*. 6-week-old SLRKO female mice displayed a trend increase in body weight ($p=0.101$) compared to fl/fl littermates (Fig. 2A). Furthermore, SLRKO female mice exhibited increased iBAT, iWAT, and gWAT fat mass (Fig. 2B) with larger adipocyte size (Fig. 2C). This difference in fat pad mass and body weight was due to decreased energy expenditure evident by reduced oxygen consumption and heat production (Fig. 2D-E), as there was no difference in food intake (Fig. 2F). There were no noticeable differences in locomotor activity (Supplementary Fig. 2D) or gene expression in the gastrocnemius muscle between animal groups (Supplementary Fig. 2C). This indicates that the observed decrease in energy expenditure of SLRKO female mice is likely due to deficiencies in the thermogenic capacity of adipose tissue. Indeed, SLRKO females exhibit downregulated expression of several thermogenic genes such as uncoupling protein 1 (UCP1), peroxisome proliferator-activated receptor-gamma-1 alpha ($Pgc1\alpha$), and iodothyronine deiodinase 2 (DIO2) in both iBAT and iWAT (Fig. 3A & 3C). Furthermore, we observed a trend decrease ($p=0.061$) in Atp2a2 expressions levels (Fig. 3A). The Atp2a2 gene encodes the Ca^{2+} -ATPase2b (SERCA2b) pump that has recently been shown to regulate UCP1-independent beige

adipocyte thermogenesis (Ikeda et al., 2017). Consistently, UCP1 protein levels are reduced in iWAT evident by a decrease in UCP1 staining (Fig. 3E) indicating decrease in the abundance of thermogenic beige adipocytes within the iWAT depot. On the other hand, UCP1 protein levels were not reduced in iBAT of SLRKO female mice (Fig. 3D). Interestingly, the observed decrease in beige adipocytes, overall thermogenic capacity of iWAT and iBAT, and decrease in whole-body energy expenditure is correlated with a significant decrease in TH protein levels in iWAT and trend decrease in iBAT ($p=0.086$) (Fig. 3B & 3D). This potentially indicates that the observed decrease in whole-body energy expenditure in SLRKO female mice may be due to decreased sympathetic innervation of adipose tissue. This is further supported by the upregulation of B3AR in iWAT (Fig. 3A), potentially indicating a feedback mechanism increasing B3AR expression due to decreased sympathetic innervation and norepinephrine (NE) levels in iWAT.

Interestingly, 6-week-old SLRKO male mice did not exhibit the same metabolic phenotype as females. There was no noticeable difference in body weight (Fig. 4A) or fat pad mass (Fig. 4B). Furthermore, SLRKO males did not exhibit any differences in oxygen consumption (Fig. 4C), energy expenditure (Fig. 4D), food intake (Fig. 4E), and locomotor activity (Supplementary Fig. 3D). The lack of phenotypic differences was not due to lower sympathetic-specific LepR KO efficiency as LepR expression levels were reduced by ~ 40%, similar to SLRKO females (Supplementary Fig. 3A-3B). Surprisingly, SLRKO males exhibited a significant downregulation of the thermogenic genes UCP1, DIO2, Cidea in iWAT (Fig. 4F). However, we did not observe a difference in UCP1 protein levels in iWAT (Fig. 4H). In iBAT, however, there were no changes in thermogenic gene expression (Fig. 4G and Supplementary Fig. 3E). We hypothesize that the phenotypic and gene expression differences between males

and females is due to mild cold stress induced thermogenesis, since male mice experience cold-induced thermogenesis at lower ambient temperatures than females (Gomez-Garcia et al., 2022).

Metabolic characterization at thermoneutral conditions is necessary to confirm this hypothesis.



*Figure 2. 6-week-old female mice with LepR deficiency in sympathetic neurons exhibit decreased energy expenditure and increased adiposity. (A) Body weight at the time of analysis ($n = 6$). (B) Tissue weights ($n = 6$). (C) H&E staining of iWAT and BAT (iWAT $n = 4$ for both groups, BAT $n = 3$ for both groups). (D) Oxygen consumption ($n = 6$ for both groups; Light phase represented by white background; Dark phase represented by grey background). (E) Energy Expenditure measured as heat production ($n = 6$ for both groups; Light phase represented by white background; Dark phase represented by grey background). (F) Food intake measurement of 6-week-old females, measurements recorded during the animal's 6th week of life ($n = 6$ for both groups). All data are expressed as mean \pm SEM; * $p < 0.05$.*

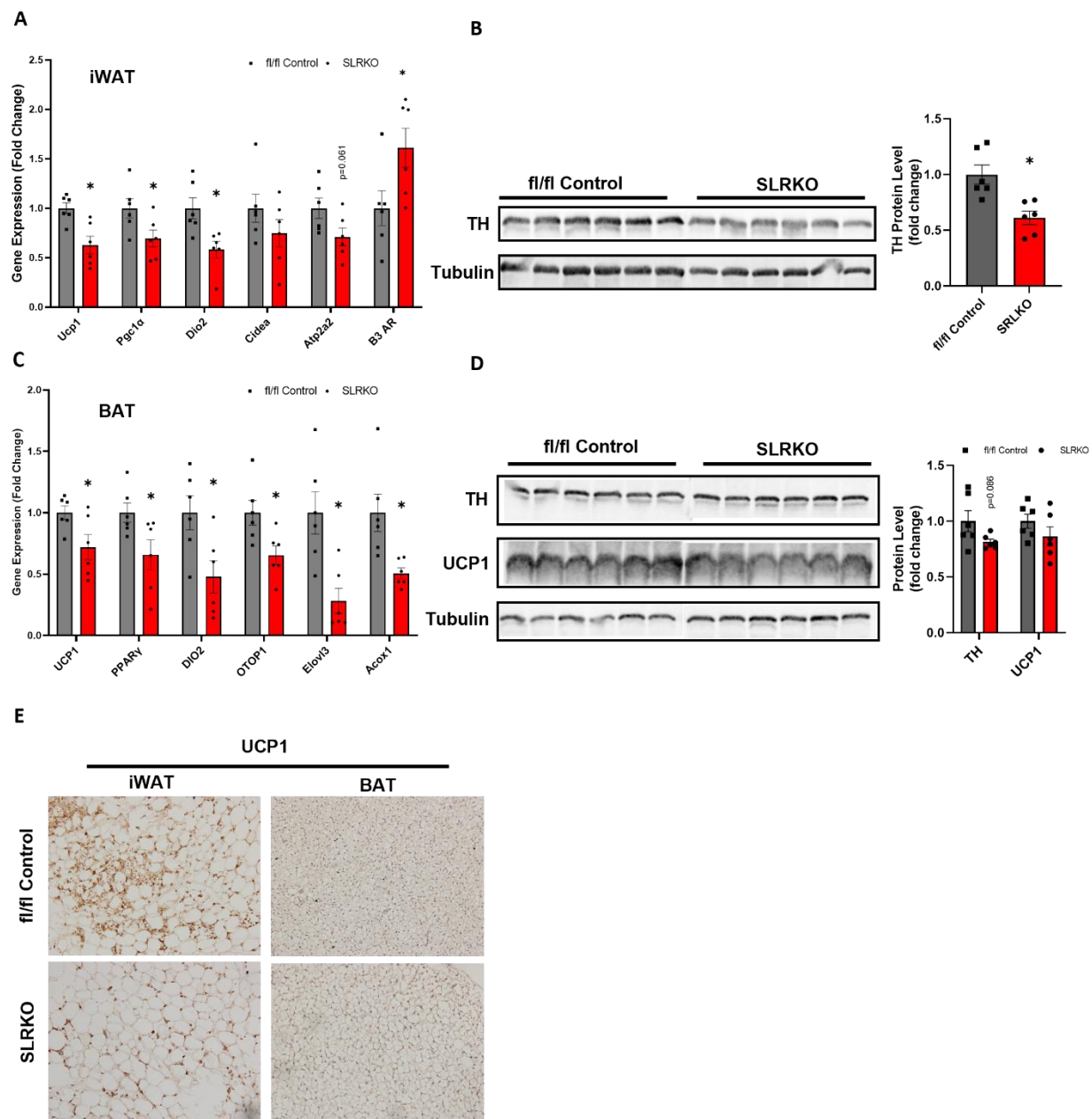


Figure 3. 6-week-old female mice with *LepR* deficiency in sympathetic neurons exhibit decreased thermogenic gene expression and TH protein levels in iWAT and BAT. (A) Quantitative RT-PCR analysis of thermogenic gene expression in iWAT ($n = 5-6$ per group). (B) TH protein content in iWAT ($n = 6$). (C) Quantitative RT-PCR analysis of thermogenic gene expression in BAT ($n = 5-6$ per group). (D) TH and UCP1 protein content in BAT ($n = 6$). (E) UCP1 staining in iWAT and BAT (iWAT $n = 3$, BAT $n = 4$). All data are expressed as mean \pm SEM; * $p < 0.05$.

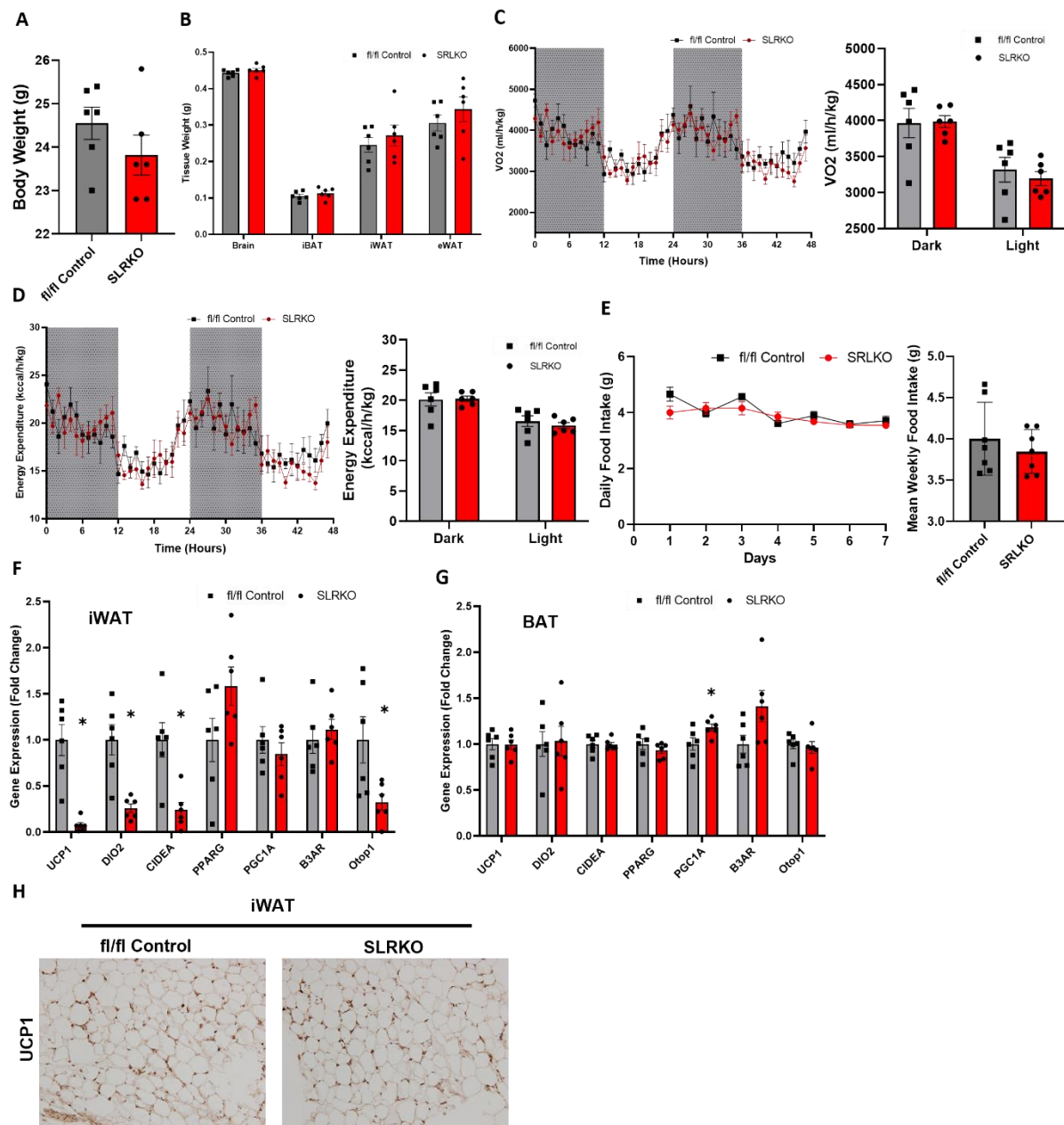


Figure 4. 6-week-old male mice with *LepR* deficiency in sympathetic neurons display decreased iWAT thermogenic gene expression with no associated metabolic phenotype. (A) Body weight at the time of analysis ($n = 6$). (B) Tissue weights ($n = 6$). (C) Oxygen consumption ($n = 6$ for both groups; Light phase represented by white background; Dark phase represented by grey background). (D) Energy Expenditure measured as heat production ($n = 6$ for both groups; Light phase represented by white background; Dark phase represented by grey background). (E) Food intake measurement of 6-week-old males, measurements recorded during the animal's 6th week of life ($n = 6$ for both groups). (F) Quantitative RT-PCR analysis of thermogenic gene expression in iWAT ($n = 5-6$ per group). (G) Quantitative RT-PCR analysis of thermogenic gene expression in BAT ($n = 6$ per group). (H) UCP1 staining of iWAT ($n = 2$ for both groups). All data are expressed as mean \pm SEM; * $p < 0.05$.

4.3 Female Mice with Sympathetic Neuron Specific Deletion of *LepR* are Prone to Diet-Induced Obesity

The observed increase in fat mass and decrease in energy expenditure led us to hypothesize that SLRKO mice would be more prone to the development of diet-induced obesity (DIO). Indeed, we discovered that a high fat diet (HFD) consisting of 60% calories from fat markedly increased body weight in SLRKO females when compared to fl/fl control mice (Fig. 5A). Interestingly, SLRKO females were already significantly heavier at 6-weeks of age before HFD feeding, but the body weight difference was further exacerbated after 30 weeks on HFD (Fig. 5A). By using a Minispec NMR body composition analyzer, we also observed a significant increase in overall fat mass coupled with a decrease in overall lean mass (Fig. 5B). Tissue analysis revealed an increase in iBAT, iWAT and gWAT mass (Fig. 5C). Histological analysis in the form of H&E staining revealed an increase in adipocyte size in both iWAT and iBAT of SLRKO females compared to fl/fl controls (Fig. 5D). We next measured food intake and energy expenditure to understand the underlying cause of the difference in body weight and fat mass. Similar to chow-fed 6-week-old mice, HFD-fed SLRKO and fl/fl control mice have comparable food intake (Fig. 5G), *LepR* and neuropeptide expression in the ARC (Supplementary Fig. 4A). However, SLRKO mice displayed reduced energy expenditure when compared to fl/fl controls in the dark and light phases (Fig. 5E-5F), suggesting that sympathetic *LepR* KO affects energy expenditure but not food intake in female mice. Furthermore, SLRKO females did not exhibit differences in locomotor activity (Supplementary Fig. 4B). As obesity can lead to Type 2 Diabetes development in humans and mice, we explored the glucoregulatory ability of HFD-fed SLRKO and fl/fl mice. We observed a mild difference in circulating glucose levels at 45 and 60

minutes following an insulin tolerance test (ITT) (Supplementary Fig. 4C). However, sympathetic LepR deletion did not alter glucose disposal during a glucose tolerance test (GTT) (Supplementary Fig. 4D). Lastly, there were no differences in the respiratory exchange ratio (RER) between SLRKO and fl/fl controls (Supplementary Fig. 4E), indicating no difference in the preferential utilization of glucose or fatty acids as fuel.

We then hypothesized that the DIO susceptibility and reduced energy expenditure in SLRKO female mice was due to decreased thermogenic capacity of iWAT and iBAT, similar to what was observed in chow-fed 6-week-old mice (Fig. 2C-2E and Fig. 3). Indeed, we discovered a significant reduction in thermogenic genes such as UCP1, DIO2, Atp2a2, and Cidea in iWAT of SLRKO female mice (Fig. 6A), while only DIO2 was downregulated in iBAT (Fig. 6C). Moreover, we found a trend decrease of TH protein content ($p=0.0784$) in SLRKO iWAT compared to fl/fl littermates (Fig. 6B). We did not observe differences in TH or UCP1 proteins levels in SLRKO iBAT compared to fl/fl controls (Fig. 6D). Together, these data suggest that the increased DIO susceptibility and decreased energy expenditure in SLRKO females is primarily due to decreased thermogenic capacity and SNS innervation of iWAT.

Like chow-fed males, HFD-fed SLRKO males did not exhibit a body weight difference compared to fl/fl littermates (Fig. 7A). Interestingly, SLRKO males exhibited a decrease in fat mass (Fig. 7B) reflected by a significant decrease in iBAT tissue weight (Fig. 7C) coupled with no difference in lean body mass (Fig. 7B). H&E staining revealed no abnormalities in adipocyte morphology in both iWAT and iBAT of SLRKO male mice (Fig. 7D). Moreover, SLRKO males displayed a mild decrease in oxygen consumption (Fig. 7E) and energy expenditure (Fig. 7F). This decrease was significant only during the dark phase (Fig. 7E-F) when mice are more active. Locomotor activity, however, was unchanged between SLRKO and fl/fl male mice

(Supplementary Fig. 5A). We next examined adipose tissue gene expression with a continued focus on thermogenic genes as these were downregulated in chow-fed 6-week-old males and females and HFD-fed females. Within iWAT, only epithelial V-like antigen 1 (EVA1) was downregulated (Fig. 8A), while in iBAT we observed a mild upregulation in Cidea (Fig. 8C). TH protein levels were only mildly decreased by 20% in the iWAT of SLRKO males, although not statistically significant ($p=0.19$) (Fig. 8B). In addition, there were no differences in TH and UCP1 protein levels in iBAT (Fig. 8D). We next explored any potential glucoregulatory differences between animal groups. Surprisingly, SLRKO males showed more pronounced glucose intolerance and insulin resistance when compared to fl/fl littermates (Supplementary Fig. 5C-5D), even though they displayed no difference in body weight (Fig. 7A) and a mild decrease in adiposity (Fig. 7B). This may be due to a decrease in iBAT weight (Fig. 7C) and potential changes in glucose utilization in iBAT as no meaningful differences in gastric muscle mRNA levels were observed (Supplemental Fig. 5B).

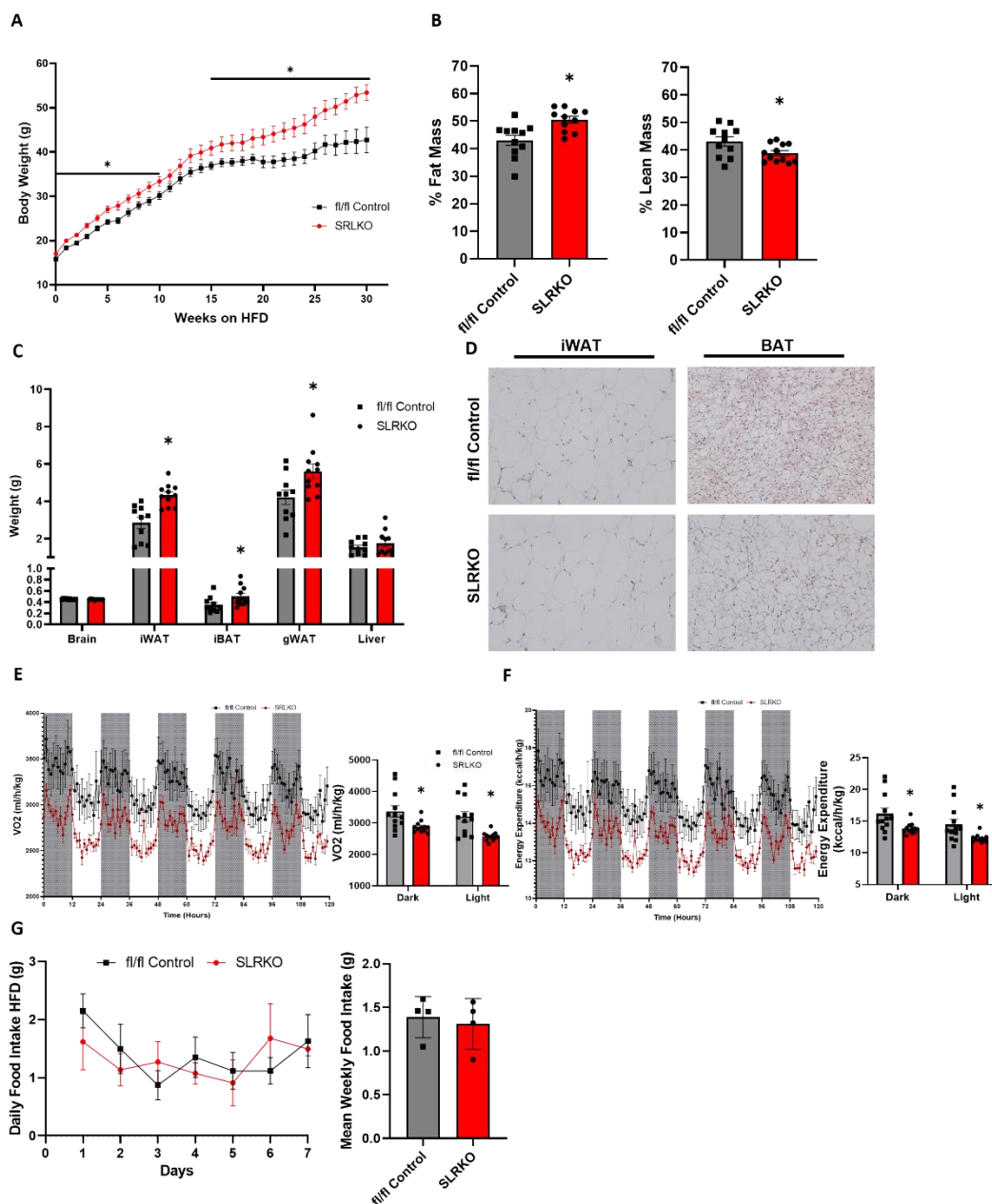


Figure 5. Female mice with sympathetic neuron specific *LepR* deletion are prone to diet-induced obesity due to decreased energy expenditure. Female SLRKO and fl/fl littermates were placed on HFD for 30 weeks when they were 6 weeks of age. (A) Body weight curve ($n = 12$ for both groups). (B) Body composition (fat mass $n = 11$ fl/fl, $n = 12$ SLRKO; lean mass $n = 11$ fl/fl, $n = 12$ SLRKO). (C) Tissue weight ($n = 10$ for both groups). (D) H&E staining for iWAT and BAT (WAT & BAT $n = 3$ for both groups). (E) Oxygen consumption ($n = 12$ for both groups; Light phase represented by white background; Dark phase represented by grey background). (F) Energy Expenditure measured as heat production ($n = 12$ for both groups; Light phase represented by white background; Dark phase represented by grey background). (G) Food intake measurement of females during week 24 on HFD, measurements recorded during the animal's 30th week of life ($n = 4$ for both groups). All data are expressed as mean \pm SEM; * $p < 0.05$.

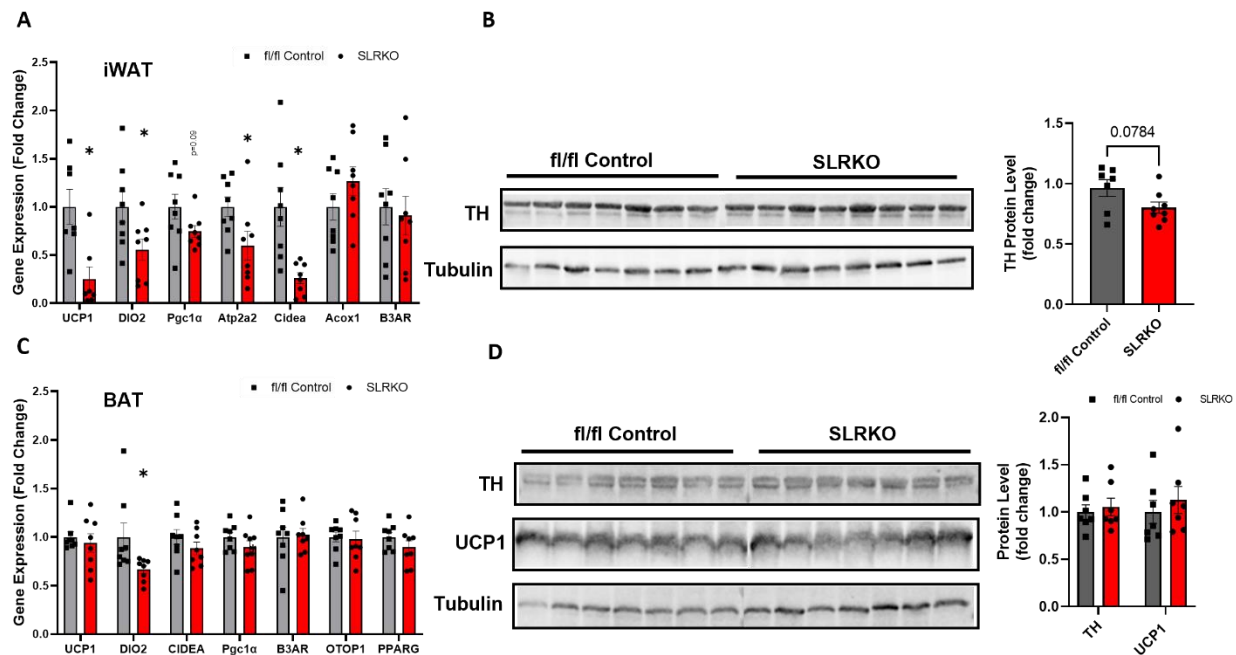


Figure 6. HFD-fed female mice with sympathetic neuron specific *LepR* deletion exhibit decreased expression of thermogenic genes and TH protein content in iWAT. (A) Quantitative RT-PCR analysis of thermogenic gene expression in iWAT ($n = 7-8$ per group). (B) TH protein content in iWAT (fl/fl $n = 7$, SLRKO $n = 8$). (C) Quantitative RT-PCR analysis of thermogenic gene expression in BAT ($n = 7-8$ per group). (D) TH and UCP1 protein content in BAT ($n = 7$). All data are expressed as mean \pm SEM; * $p < 0.05$.

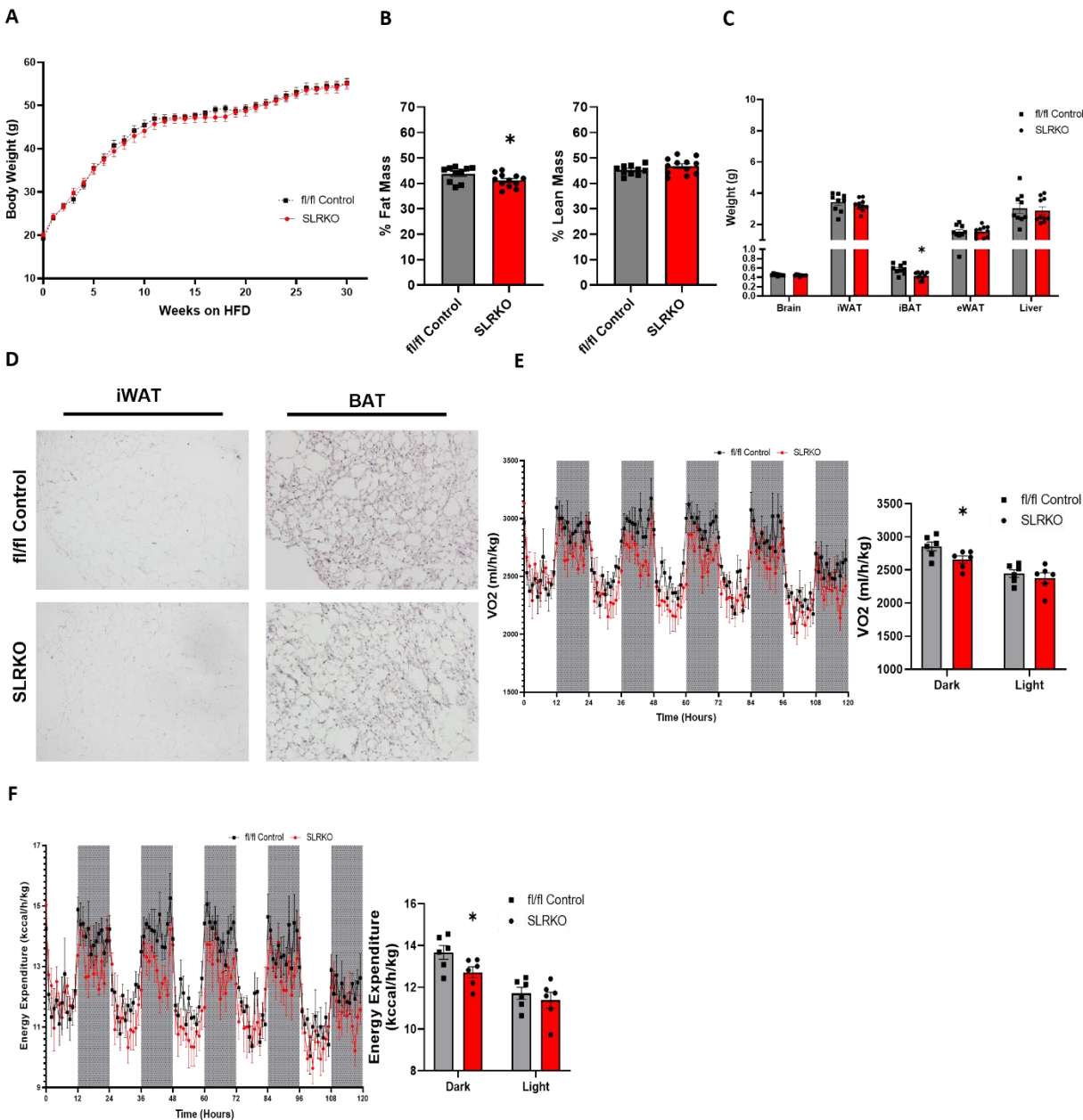
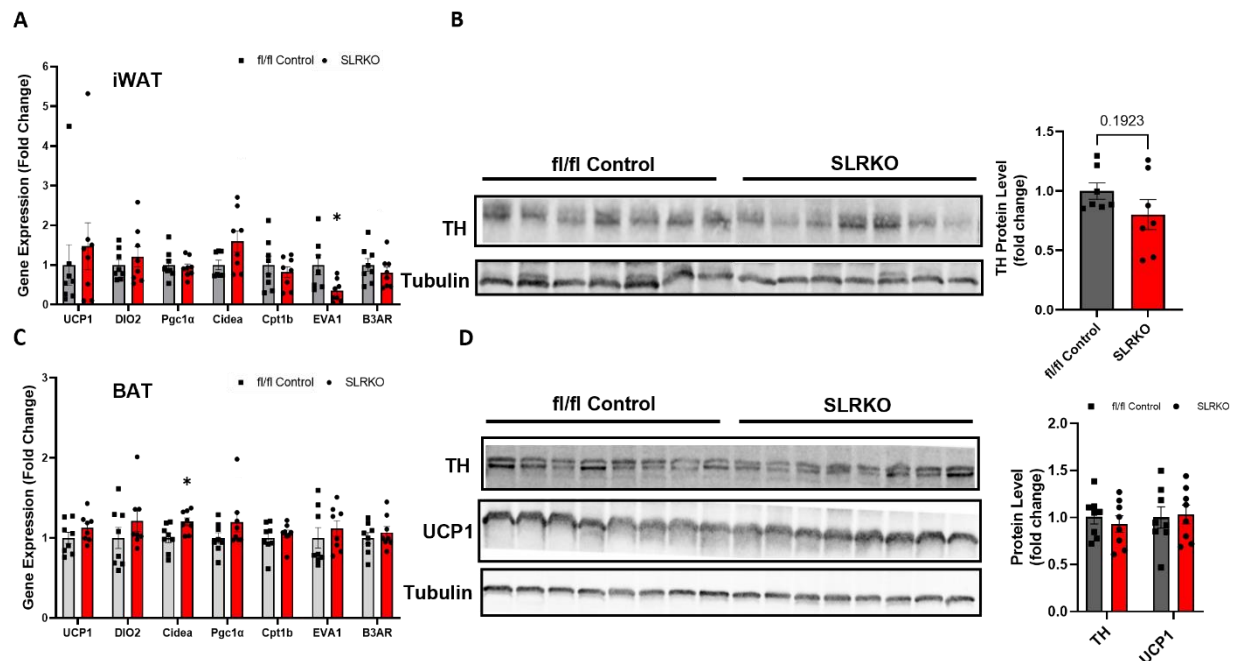


Figure 7. Male mice with sympathetic neuron specific *LepR* deletion exhibit decreased energy expenditure and a decrease in BAT weight. Male SLRKO and *fl/fl* littermates were placed on HFD for 30 weeks when they were 6 weeks of age. (A) Body weight curve (*fl/fl* $n = 10$, SLRKO $n = 12$). (B) Body composition (*fl/fl* $n = 10$, SLRKO $n = 12$). (C) Tissue weight (*fl/fl* $n = 10$, SLRKO $n = 12$). (D) H&E staining for iWAT and BAT (WAT & BAT $n = 3$ for both groups). (E) Oxygen consumption (*fl/fl* $n = 10$, SLRKO $n = 12$; Light phase represented by white background; Dark phase represented by grey background). (F) Energy Expenditure measured as heat production (*fl/fl* $n = 10$, SLRKO $n = 12$; Light phase represented by white background; Dark phase represented by grey background). All data are expressed as mean \pm SEM; * $p < 0.05$.



*Figure 8. HFD-fed male mice with sympathetic neuron specific LepR deletion exhibit no changes in thermogenic gene expression. (A) Quantitative RT-PCR analysis of thermogenic gene expression in iWAT (n = 7-8 per group). (B) TH protein content in iWAT (fl/fl n = 7, SLRKO n = 7). (C) Quantitative RT-PCR analysis of thermogenic gene expression in BAT (n = 7-8 per group). (D) TH and UCP1 protein content in BAT (n = 8). All data are expressed as mean \pm SEM; * $p < 0.05$.*

4.4 LepR Signaling Regulates Diet-Induced Thermogenesis but Not Cold-Induced Thermogenesis in Adult Mice

To investigate the role of sympathetic neuron leptin-LepR signaling in cold-induced thermogenesis, we placed adult SLRKO mice (Females: 6-month-old $n = 7$, 4-month-old $n = 1$; Males: 6-months-old $n = 6$) and fl/fl littermates (Females: 6-month-old $n = 6$, 4-month-old $n = 2$; Males 6-months-old $n = 7$) in a chronic 7-day 4°C cold challenge. Unexpectedly, neither male nor female SLRKO mice exhibited any differences in iWAT, eWAT, or iBAT fat pad mass compared to fl/fl controls (Supplementary Fig. 6A-6B). Furthermore, thermogenic gene expression analysis showed no differences in mRNA expression between SLRKO and fl/fl animals in both iWAT and iBAT (Supplementary Fig. 6C-6D). To explore the reason behind this apparent lack of differential phenotype between SLRKO and fl/fl mice, we measured serum leptin levels in WT adult male mice (6-month-old) after varying lengths of cold exposure (Supplementary Fig. 6E). We observed that adult mice experienced a steady decrease in serum leptin content the longer they were exposed to a cold environment (4°C). This steady decrease reached statistical significance after 7 days at 4°C (Supplementary Fig. 6E). This contrasted with what we previously observed in younger 6-week-old WT mice. Younger mice displayed a surge in serum leptin after 1 day at 4°C, which eventually decreased back to baseline levels after 7 days at 4°C (Fig. 1C). We also measured iWAT and iBAT leptin mRNA levels in adult WT mice (Supplementary Fig. 6F-G). Leptin expression was unchanged in iWAT and was greatly decreased in iBAT after 6 hours or 1 day at 4°C (Fig. 6F-G). In contrast, 6-week-old WT mice experienced a 10- and 5-fold increase in iWAT leptin expression after 1 day and 7 days at 4°C, respectively (Fig. 1D). Cold-induced changes in iBAT leptin expression were similar in adult

and 6-week-old WT mice. Both groups experienced a downregulation of leptin expression, although this downregulation appears to be more pronounced in adult mice (Supplementary Fig. 6G and Fig. 1E). The lack of an increase in serum protein and iWAT mRNA leptin levels may lead to a lack of leptin-induced thermogenesis in aging WT mice, which may explain why we did not observe a difference in thermogenic gene expression between SLRKO and fl/fl controls. Furthermore, the serum leptin content of adult mice was significantly higher than that of 6-week-old mice while housed at room temperature (RT) (Supplementary Fig. 6H). This is likely due to the adult mice having a larger adipose tissue organ than 6-week-old mice, as serum leptin level is directly proportional to adipose tissue size. The higher RT leptin levels may potentially indicate that the adult animals may be leptin resistant. Leptin resistance would result in decreased leptin signaling and potentially a decrease in leptin-induced thermogenesis in WT mice, which could further explain why there was no difference in thermogenic capacity between adult SLRKO and fl/fl mice during a cold challenge. However, future studies looking at cold challenged 6-week-old mice are necessary to fully elucidate the role of sympathetic leptin-LepR signaling in cold-induced thermogenesis.

We next examined if sympathetic leptin-LepR signaling regulates diet-induced thermogenesis. To this end, we measured oxygen consumption and energy expenditure in SLRKO and fl/fl control female mice that underwent a 16-hour fast followed by a 24-hour re-feeding period. Furthermore, these mice were housed in thermoneutral conditions (30 °C) to avoid adaptive thermogenesis that may be induced by mild cold stress in ambient temperature environments (20-24°C) (Cui et al., 2016). When housed in thermoneutral conditions and allowed *ad lib* access to HFD, SLRKO females exhibited significantly decreased oxygen consumption and energy expenditure compared to fl/fl controls. However, this difference was no

longer statistically significant during a 16 hour fast (Fig. 9A-9B). SLRKO females once again exhibited significantly decreased oxygen consumption and energy expenditure during the re-feeding period (Fig. 9A-9B, see section labeled “Re-feed”). Since mice are most active and consume 70% their daily calories during the dark phase of the light/dark cycle (Ellacott et al., 2010) and to further assess the impact of feeding on energy expenditure, we compared oxygen consumption and energy expenditure between the dark phases of each feeding period (Fast, Re-feed, *Ad Lib*) within fl/fl and SLRKO animal groups. Indeed, fl/fl females exhibited a significant increase in oxygen consumption and energy expenditure during the re-feed dark and *ad lib* dark feeding periods compared to the dark phase fasting period (Fig. 9C-D). This indicates that fl/fl females experience diet-induced thermogenesis, since their energy expenditure is significantly higher in feeding periods compared with fasting. Interestingly, SLRKO females did not display any differences in oxygen consumption or energy expenditure between these three feeding periods (Fig. 9C-D). This indicates that SLRKO females did not experience diet-induced thermogenesis as feeding did not increase energy expenditure compared with fasting. Furthermore, SLRKO females had a significantly increased respiratory exchange rate (RER) during the re-feeding period indicating preferential utilization of glucose as fuel (Supplementary Fig. 7A). Since these animals were HFD-fed during the RER measurement, this observed RER increase may also be due to potentially impaired fatty acid oxidation in SLRKO mice. Taken together, our data strongly suggests that sympathetic Leptin-LepR signaling regulates diet-induced thermogenesis in female mice.

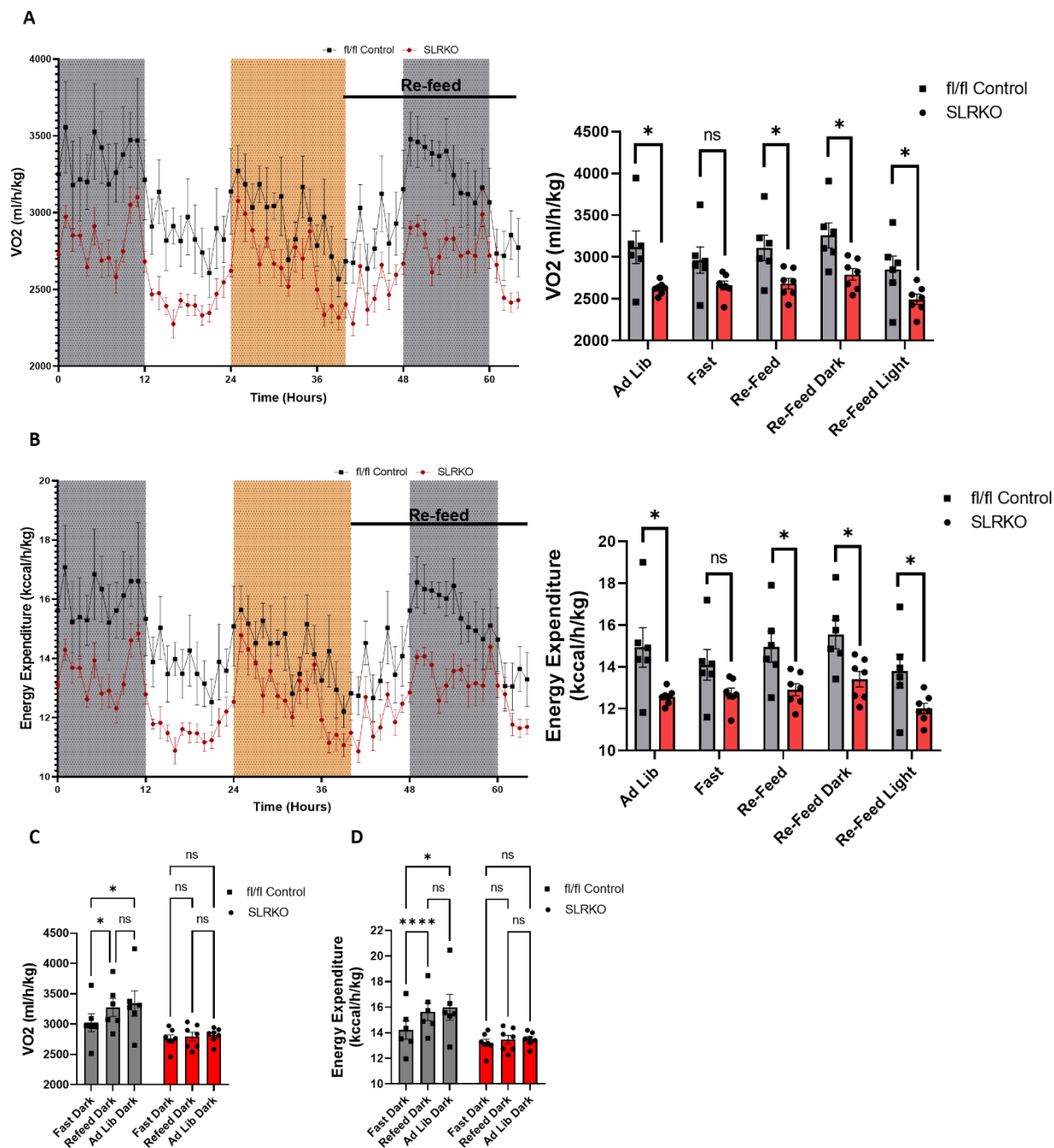


Figure 9. HFD-fed female mice with sympathetic neuron specific *LepR* deletion exhibit impaired diet-induced thermogenesis. HFD-fed female SLRKO and *fl/fl* littermates were placed in thermoneutral conditions (30 °C) then fasted for 16 hours (represented by the gold background) and re-fed for 24 hours (light phase represented by white background; dark phase represented by the grey background). (A) oxygen consumption ($n = 6-7$). (B) Energy expenditure measured as heat production ($n = 6-7$). (C) Within groups mixed model ANOVA comparison of oxygen consumption ($n = 6-7$, multiple comparison calculated with Tukey HSD). (D) Within groups mixed model ANOVA comparison of energy expenditure ($n = 6-7$, multiple comparison calculated with Tukey HSD). All data are expressed as mean \pm SEM; * $p < 0.05$.

4.5 LepR Signaling Regulates Sympathetic Axon Growth and White Adipose Tissue Innervation

To further explore the mechanism by which leptin regulates sympathetic innervation in adipose tissue, we investigated the importance of LepR in regulating sympathetic neuron growth and iWAT innervation. Therefore, we treated L1 sympathetic neurons isolated from fl/fl or SLRKO mice with leptin and found that deleting LepR in sympathetic neurons significantly suppressed leptin-induced neurite growth (Fig. 10A, fl/fl + leptin vs. SLRKO + leptin) without affecting basal growth (Fig. 10A, fl/fl + PBS vs. SLRKO + PBS). This indicates that leptin's neurotrophic effects on sympathetic neurons require LepR signaling. Unsurprisingly, sympathetic neuron growth stimulated by trophic factors used *in vitro* such as NGF is not impaired by LepR deletion as.

Additionally, by using a modified version of the Adipo-Clear approach, we were able to render the iWAT depot for our mice transparent. This coupled with TH immunostaining and light sheet microscopy allowed us to fully visualize sympathetic nerve fibers within the iWAT. Interestingly, we observed a profound decrease of sympathetic innervation in the iWAT of 6-week-old SLRKO females when compared to fl/fl controls (Fig. 10B). Thus, our data strongly supports the importance of leptin-LepR signaling in regulating sympathetic innervation of iWAT. Furthermore, this explains the observed decrease in energy expenditure (Fig. 2F), thermogenic gene expression (Fig. 3A), TH and UCP1 protein content (Fig. 3B & 3E), and increased adiposity (Fig. 2A-C) in SLRKO female mice when compared to fl/fl controls.

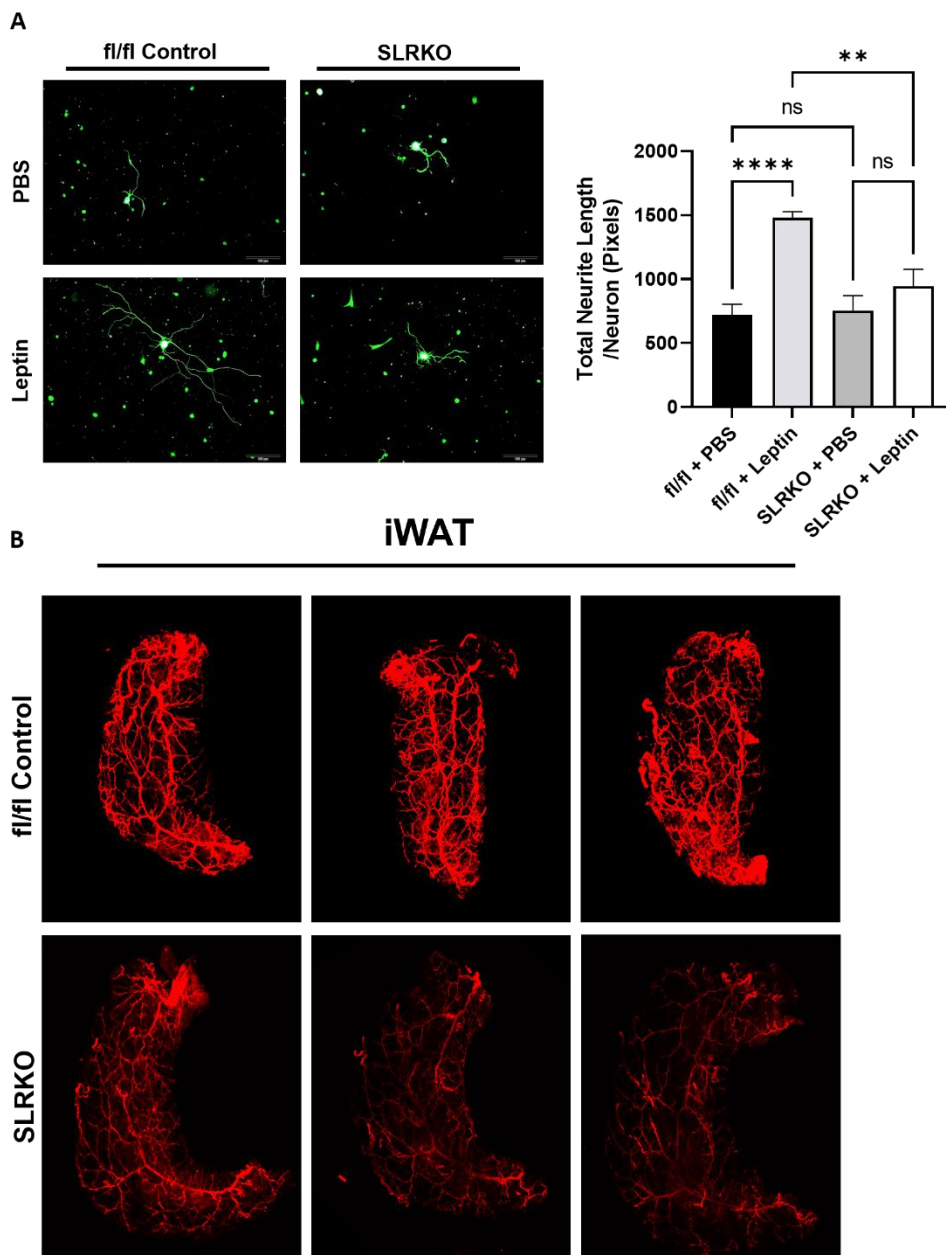


Figure 10. Sympathetic *LepR* regulates iWAT sympathetic innervation and is required for the neurotrophic effects of Leptin. (A) Representative β III-tubulin Immunofluorescent images of sympathetic ganglia neurons isolated from 6-week-old SLRKO and f1/f1 female mice treated with phosphate-buffered saline (PBS) or Leptin (100 ng/ml) and quantitation of total neurite length per neuron (f1/f1 + PBS $n = 20$, f1/f1 + Leptin $n = 16$, SLRKO + PBS $n = 16$, SLRKO + Leptin $n = 18$). Statistical significance was analyzed with two-way ANOVA (* Indicates statistical significance with Tukey's multiple comparisons test). (B) Representative images of TH-stained nerve fibers in WAT of 6-week-old chow-fed SLRKO and f1/f1 littermates. All data are expressed as mean \pm SEM; * $p < 0.05$.

4.6 LepR Regulated Mitochondrial Oxidative Metabolism is Important for Sympathetic Neuronal Growth.

To gain further insight into the underlying mechanisms governing leptin/LepR-induced neurite growth and iWAT innervation, we performed RNA-seq analysis in lumbar L1 sympathetic ganglia that innervates iWAT from 6-week-old SLRKO and fl/fl littermates. This allowed us to unbiasedly examine differentially expressed genes and genetic pathways.

Our RNA-seq analysis revealed a total of 665 differentially regulated genes between young SLRKO and fl/fl female mice ($\text{Log}_2(\text{fold change}) \geq 0.5$ or ≤ -0.5); out of which 526 were downregulated, and 139 were upregulated. Axonal outgrowth and guidance toward final innervation targets involve complex coordination of various cellular programs including axonal transport of mitochondria toward the distal axon and growth cone, ATP synthesis to power various cellular processes, and synthesis, transport, and assembly of cytoskeletal polymer protein to promote axon extension (Dent et al., 2011; Goldberg, 2003). Pathway analysis revealed several differentially regulated genetic pathways. Among them, several downregulated pathways involved in mitochondrial oxidative metabolism, ATP production, and formation of contractile fibers were observed (Fig. 11A). The primary role of mitochondria is energy production in the form of ATP. Indeed, increasing ATP synthesis via the stimulation of mitochondrial oxidative respiration results in increased peripheral nerve growth (Zhou et al., 2016). On the other hand, a reduction in mitochondrial oxidative respiration reduced axon outgrowth (Han et al., 2016). Interestingly, STAT3 overexpression increases both mitochondrial oxidative respiration and retinal ganglion axon regeneration after optic nerve injury (Luo et al., 2016). Since leptin is a strong activator of STAT3 via LepRb signaling (Vaisse et al., 1996), LepRb deletion in

sympathetic neurons could explain the observed downregulation in mitochondrial oxidation gene and decrease in sympathetic axon growth (Fig. 10A-B). Our heatmap analysis (Fig. 11B) and volcano plot (Fig. 11C) revealed several downregulated mitochondrial oxidation genes, including several subunits of the mitochondrial ATP synthase (*Atp5a1*, *Atp5b*, *Atp5c1*, *Atp5pb*, *Atp5g1*, and *Atp5j*), several subunits of mitochondrial cytochrome c oxidase (COX) (*Cox4i1*, *Cox5a*, *Cox5b*, *Cox6a1*, *Cox6a2*, *Cox6c*, *Cox7a1*, *Cox7a2*, *Cox7c*, *Cox8a*, and *Cox8b*), and 4 subunits of mitochondrial complex 1 NADH: ubiquinone oxidoreductase (*Ndufa2*, *Ndufa4*, *Ndufs4*, and *Nduv1*) (Fig. 11B-C). The downregulation of these genes is indicative of robust perturbations in mitochondrial oxidative metabolism and ATP production. A decrease in ATP production and availability is a potential mechanism for the suppressed sympathetic axon growth and iWAT innervation that we observed in our animals with sympathetic specific LepR deletion.

Furthermore, transglutaminase 2 (*Tgm2*) and transglutaminase 3 (*Tgm3*) were downregulated in SLRKO female mice. Transglutaminase 2 has been shown to act as a calcium-dependent transmediating enzyme, GTPase, disulfide isomerase, and most recently a potent transcription factor regulating neuron viability and neurite growth (Begg et al., 2006; Gundemir et al., 2011; Yunes-Medina et al., 2018). Indeed, neuronal depletion of *Tgm2* results in greatly decreased total neurite length and neuron viability in rodent cortical neurons (Yunes-Medina et al., 2018). We also found several myosins and troponins downregulated in SLRKO female mice, including myosin heavy chain 1 (*Myh1*), myosin light chain 1 (*My11*), myosin light chain 6 (*My16*), troponin C2 (*Tnnc2*), troponin I (*Tnni2*), troponin T2 (*Tnnt2*) (Fig. 11B-C). Although these genes are primarily associated with muscle contraction, *Myh1* downregulation has recently been implicated in the development of the neurodegenerative disease Amyotrophic lateral sclerosis (ALS) (Xu et al., 2019). Thus, these various myosin components may be

previously unidentified components of axonal cytoskeletal proteins that are regulated by Leptin-LepR signaling and are of importance for sympathetic axon growth and innervation of adipose tissue.

Lastly, we observed the upregulation of several protocadherin (*Pcdh*) genes, which regulate intercellular adhesion and cell-cell communications (Fig. 11C). These genes include, protocadherin 1 (*Pcdh1*), protocadherin alpha 7 (*Pcdha7*), protocadherin gamma subfamily B 4 (*Pcdhgb4*), protocadherin gamma subfamily B 2 (*Pcdhgbb2*), and protocadherin gamma subfamily A 7 (*Pcdhga7*) (Fig. 11C). Protocadherins exist as 3 linked gene clusters, *Pcdh* α (*Pcdha*), *Pcdh* β (*Pcdhb*), and *Pcdh* γ (*Pcdhg*) (Chen & Maniatis., 2013). Although protocadherins are expected to promote axon growth and target tissue innervation within the brain (Pancho et al., 2020), differential overexpression of protocadherin clusters such as *Pcdhg* and *Pcdha* (as observed in this study) have been shown to reduce olfactory sensory neuron axon convergence and formation of glomeruli (Mountoufaris et al., 2017). Additionally, forebrain serotonergic neurons express the same *Pcdh* α -c isoform, which mediates axonal repulsion ensuring even serotonergic axon distribution in the forebrain (Chen et al., 2017). Therefore, the observed decrease in sympathetic adipose tissue innervation could partially be due to contact mediated sympathetic axon repulsion between axons overexpressing identical combinations of *Pcdh* isoforms. However, the mechanism by which leptin signaling regulates differential expression of protocadherins is still unknown.

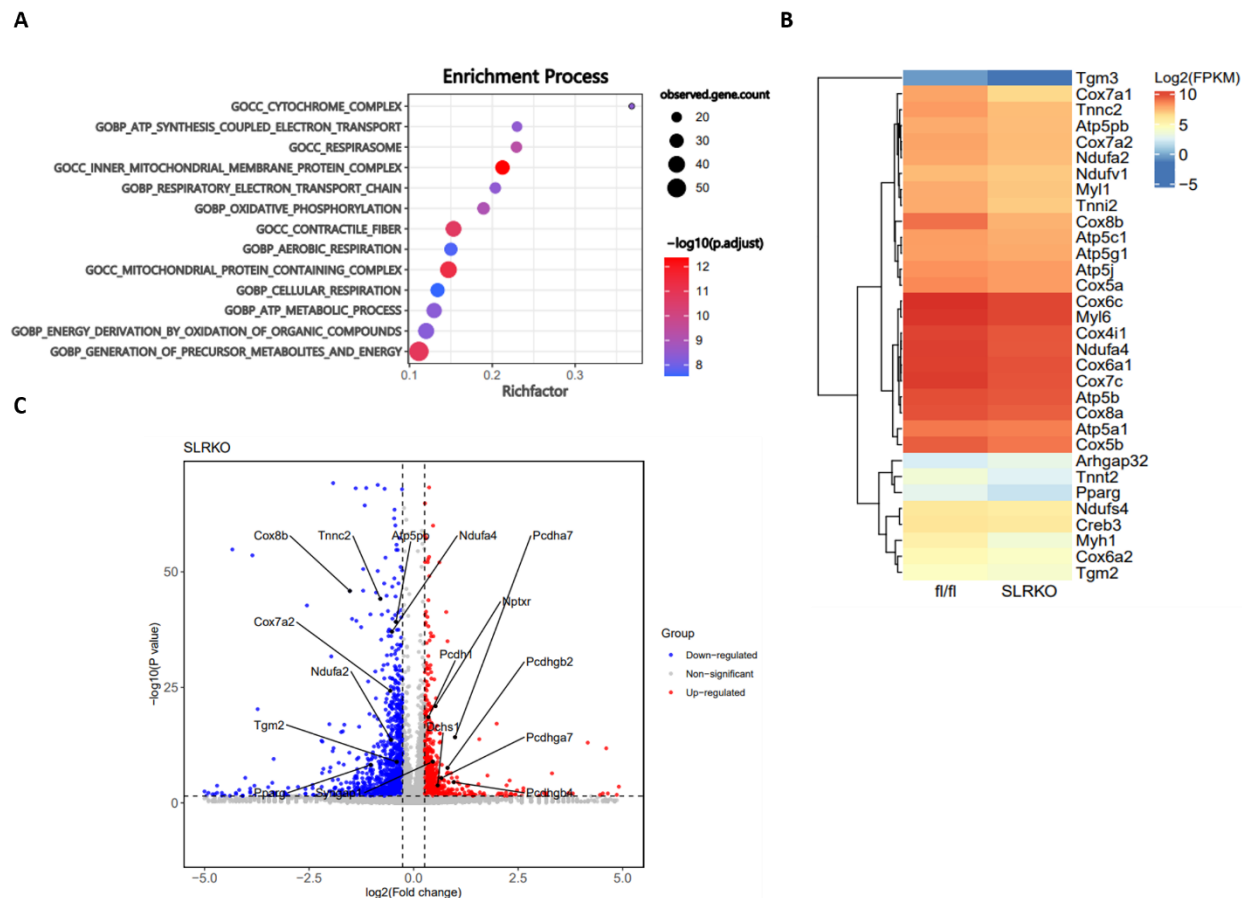


Figure 11. RNA analysis of sympathetic ganglia from 6-week-old SLRKO and fl/fl females reveals defects in mitochondrial oxidative metabolism. L1 sympathetic ganglia RNA from SLRKO (n = 8) and fl/fl (n = 7) mice were diluted to equal concentrations and pooled into 2 samples, respectively. (A) GO pathway analysis of downregulated genes in SLRKO vs. fl/fl female mice. (B) Heatmap of the downregulated genes in SLRKO female mice compared to fl/fl littermates. (C) Volcano plot of differentially expressed genes involved in mitochondrial oxidative metabolism, contractile fiber function, and synaptogenesis (Log_2 fold change ≥ 0.5)

5 DISCUSSION

The global prevalence of obesity continues to rise at an increasingly alarming rate. Multiple countries have reported a two to three-fold increase in the prevalence of obesity in the last three decades alone (Tiwari & Balasundaram, 2023). In the United States, 41.9% of adults aged 20 or older and 19.7% of children and adolescents aged 2-19 are considered obese (BMI \geq 30 Kg/m²) based on a national health and nutrition examination survey conducted from 2017 to March 2020 (Stierman et al., 2021). Furthermore, the obesity prevalence in the United States is expected to have significantly increased since March 2020 due to the Covid-19 pandemic and government-regulated lockdowns (Nour & Altintas, 2023). Obesity is a major risk factor for the subsequent development of various obesity-related metabolic disorders such as insulin resistance/Type 2 diabetes, hypertension, dyslipidemia, cardiovascular diseases, and some types of cancer (Hill et al., 2012). Therefore, obesity prevention and reversal are critically important in controlling the prevalence of obesity-related disorders. The dramatic increase in obesity rates is due, in part, to an increasingly sedentary lifestyle resulting in decreased energy expenditure coupled with an increase in the consumption of high calorie processed foods resulting in increased calorie intake. Simply stated, obesity is observed when energy intake exceeds energy expenditure. There are various metabolic and genetic factors that contribute to this imbalance in energy metabolism (Yang et al., 2022). Hence, a comprehensive interrogation of genetic and molecular mechanisms governing the regulation of energy homeostasis is critical in our efforts to combat the escalating obesity epidemic.

Leptin, an anorexigenic adipokine primarily recognized for its role in energy homeostasis, targets the hypothalamus orchestrating metabolic regulation. Besides regulating

long term food intake and energy expenditure (Collins et al., 1996), leptin also regulates energy metabolism post-prandially by acutely increasing thermogenesis in rodents (Perry et al., 2020). Leptin acts on several hypothalamic nuclei like the ARC, DMH, VMH, and LH to influence feeding behavior and energy expenditure. Leptin's actions on ARC neurons—AGRP/NPY and POMC—exemplify its role in regulating food intake. Leptin hyperpolarizes AGRP/NPY neurons while depolarizing POMC neurons, resulting in a decrease in feeding (Qiu et al., 2010; Shanley et al., 2022). Beyond the relatively well-understood effects of leptin on feeding regulation, leptin's role in energy expenditure, particularly in promoting fat utilization and thermogenesis, has been evident. Several studies have continued to highlight the importance of hypothalamic leptin signaling in the DMH, VMH, and ARC in the regulation of energy expenditure. Interestingly, most of these studies conclude that hypothalamic leptin signaling relies on downstream SNS activation to enact the observed effects on energy expenditure, primarily by targeting metabolic tissues such as adipose tissue.

Central signals capable of activating the SNS driving iBAT and beige adipocyte thermogenesis have been a major research focus (Muenzberg et al., 2016). However, several studies have also shown that peripheral signals are able to influence SNS activity, growth, and target tissue innervation. Various neurotrophic factors such as NGF, BDNF, and NT3 have been implicated in SNS development and innervation of adipose tissue. For instance, SNS specific deletion of the NGF receptor, TrkA, results in a significant decrease in WAT innervation (Jiang et al., 2017); LysM+ myeloid specific BDNF deletion in the SVF of adipose tissue decreases SNS nerve density and thermogenic capacity of WAT (Blaszkievicz et al., 2020); Adipocyte derived NT3 overexpression increases adipose tissue innervation and SNS specific deletion of the NT3 receptor, TrkC, decreases sympathetic adipose tissue innervation (Cui et al., 2021).

Furthermore, a iBAT-derived secretory protein, S100b regulates SNS innervation of iBAT via a calcyntenin-3 β pathway (Zeng et al., 2019). Based on these studies, it is evident that target tissue-derived signals play an important role in SNS innervation.

The role of leptin as a central neurotrophic factor has been described by various studies. Briefly, leptin deficient *ob/ob* and LepR deficient *db/db* mice exhibit a structurally distinct brain anatomy compared to WT mice (Bereiter & Jeanrenaud, 1979; 1980), reflected by a robust decrease in ARC projections to the PVH (Bouret et al., 2004). Interestingly, exogenous leptin treatment in young *ob/ob* mice restores normal ARC to PVH connections, while leptin treatment in adult *ob/ob* or *db/db* mice does not (Bouret et al., 2004). This indicates that the central neurotrophic effects exerted by leptin require LepR signaling during a developmentally critical period. A recent study has implicated leptin signaling in SNS innervation of adipose tissue (Wang et al., 2020), suggesting that leptin signaling in ARC neurons projecting to BDNF-expressing neurons in the PVH regulates sympathetic postganglionic innervation of adipose tissue. The proposed top-down synaptic pathway of BDNF-releasing neurons in the PVH \rightarrow SNS preganglionic neuron \rightarrow SNS postganglionic neuron \rightarrow adipose tissue, does not fully explain how BDNF-induced modulation of preganglionic neurons in turn regulates postganglionic neuron innervation of adipose tissue (Wang et al., 2020). Furthermore, LepR expression extends beyond the central nervous system, being expressed in postganglionic sympathetic neurons (Czaja et al., 2002; Miller et al., 1999). These data indicate that SNS innervation of adipose tissue may, at least partially, rely on peripheral sympathetic leptin signaling. In this study, we demonstrate that leptin-LepR signaling in sympathetic neurons regulates SNS innervation of WAT in response to developmental cues.

In this dissertation, we employed *in vitro* leptin treatments, genetic mouse models, adipose tissue clearing, light-sheet microscopy, RNA-seq analysis, and various metabolic phenotype characterization tools to elucidate the physiological function of leptin-LepR signaling in sympathetic neurons. Our LepR-Cre::Ai14 reporter mice enabled us to visualize LepRb expression in sympathetic neurons both *in vitro* and *in vivo* that directly innervate adipose tissue. Interestingly, we also observed LepR expression in glial cells within the sympathetic ganglia. Since glial cells play an indispensable role in supporting sympathetic neuron function and growth (Hanani & Spray, 2020), future studies aiming to elucidate the functional significance of LepR signaling in sympathetic glial cells should be conducted. Metabolic characterization of our SLRKO mouse model demonstrated that sympathetic specific deletion of LepR results a significant increase in adiposity reflected by an increase in the weight of subcutaneous and visceral fat pads in chow-fed 6-week-old females. The increase in adipose tissue mass appears to be primarily due to a decrease in energy expenditure explained by an observable decrease in beige adipocyte quantity, thermogenic gene markers, and a profound decrease in sympathetic innervation of adipose tissue. Although, we found a significant decrease in several thermogenic gene markers in the iWAT of 6-week-old SLRKO males, these mice did not exhibit the increase in adiposity and decrease in energy expenditure, we observed in their female counterparts. Another difference between 6-week-old males and females lies in iBAT thermogenic gene expression. Females displayed a decrease in several iBAT thermogenic markers while males did not, implicating the role of iBAT thermogenesis in the regulation of energy expenditure in young mice. Interestingly, studies have shown that cold-induced thermogenesis is activated at higher temperatures in females (22 °C) than males (18°C) (Gomez-Garcia et al., 2022). Since these animals were housed under ambient temperatures (20-24 °C) during their metabolic

characterization, mild cold stress experienced by females but not males could account for the differences observed. Therefore, a potential impairment in cold-induced thermogenesis in 6-week-old SLRKO mice coupled with mild cold stress experienced by both 6-week-old fl/fl and SLRKO females could result in the upregulation of iBAT thermogenic genes, increasing energy expenditure in fl/fl but not in SLRKO females (Bastias-Perez et al., 2020). In contrast, the lack of mild cold stress experienced by males could explain the absence of an observable difference in energy expenditure between SLRKO and fl/fl males. However, additional studies investigating the phenotype of 6-week-old SLRKO mice under thermoneutral conditions (30°C) are needed to confirm this hypothesis.

The observed phenotype in 6-week-old SLRKO females was greatly exacerbated by HFD feeding. HFD-fed SLRKO females did not exhibit any differences in food intake but have greatly decreased energy expenditure. This resulted in the mice being significantly heavier after 30 weeks on HFD due to an increase in fat mass across all major fat depots, despite a mild decrease in lean mass. The analysis of gene expression profiles in the ARC, and VTA have ruled out the contribution of impaired leptin-LepR signaling in these nuclei in the regulation of energy homeostasis. Furthermore, we did not observe an impairment in cold induced thermogenesis but rather an impairment in diet-induced thermogenesis, as shown by fasting and re-feeding SLRKO females in thermoneutral conditions. On the other hand, HFD-fed SLRKO males did not display a difference in body weight throughout the 30 weeks on HFD. Interestingly, they did exhibit a mild decrease in energy expenditure, although no changes in thermogenic gene expression were observed. This can be explained by the observed decrease in iBAT tissue mass, since a decrease in iBAT size is accompanied by a decrease in diet-induced thermogenesis (Von Essen et al., 2017). However, the reason behind this decrease in iBAT weight is still unknown. Since iBAT

proliferation requires intact sympathetic innervation (Géloën et al., 1992), further experiments aimed at investigating the impact of sympathetic specific deletion of LepR on iBAT innervation should be conducted.

We also show that sympathetic leptin-LepR signaling directly stimulates sympathetic axon growth and is required for normal sympathetic innervation of iWAT, as seen via adipose tissue clearing coupled with light sheet microscopy. Using RNA-seq analysis, we have identified genes in several important pathways that are differentially regulated in sympathetic ganglia of 6-week-old SLRKO females compared to fl/fl controls. These include several genes involved in mitochondrial oxidative metabolism and ATP production, which is indispensable for proper axon growth, target tissue innervation, and synaptic transmission (Chamberlain & Sheng, 2019; Smith & Gallo, 2018). Indeed, mitochondrial dysfunction is seen in a plethora of neurodegenerative diseases including Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD), and Amyotrophic lateral sclerosis (ALS) (Chen & Chan, 2009; Raefsky & Mattson, 2017). Since several studies implicate STAT3 in central and peripheral axon growth/regeneration (Bareyre et al., 2011; Mehta et al., 2016; Pernet et al., 2013; Selvaraj et al., 2012), and mitochondrial metabolism in neurons (Su et al., 2020; Zhou & Too, 2011) and other cell types (Szczepanek et al., 2011; Wegrzyn et al., 2009), we believe that leptin-LepR induced STAT3 activation is an important regulator of sympathetic neuron growth. Although we expect STAT3 phosphorylation levels to be decreased in LepR deficient sympathetic ganglia, further experiments are needed to test this hypothesis. We also saw a downregulation in several genes involved in cytoskeletal composition, promoting shape change and locomotion during sympathetic nerve growth. Lastly, we saw an upregulation in several protocadherins of the *Pcdhy* gene cluster potentially leading to contact mediated repulsion of sympathetic axons.

6 CONCLUSION

In summary, this dissertation serves as a significant step forward in our understanding of how leptin regulates whole-body energy homeostasis with a focus on peripheral sympathetic leptin signaling. We have discovered a novel mechanism in which sympathetic leptin-LepR signaling regulates SNS growth and innervation of adipose tissue (Fig. 12) Our data indicates sympathetic leptin signaling may be required for proper SNS innervation in young mice and for the regulation of diet-induced thermogenesis in adult mice. Additional experiments aimed at establishing a concrete link between leptin-LepR induced STAT3 activation and sympathetic nerve growth are currently being conducted. Further studies aimed at investigating the role of sympathetic leptin signaling in cold-induced thermogenesis and sexual dimorphism in whole body energy homeostasis should be conducted to build upon our findings.

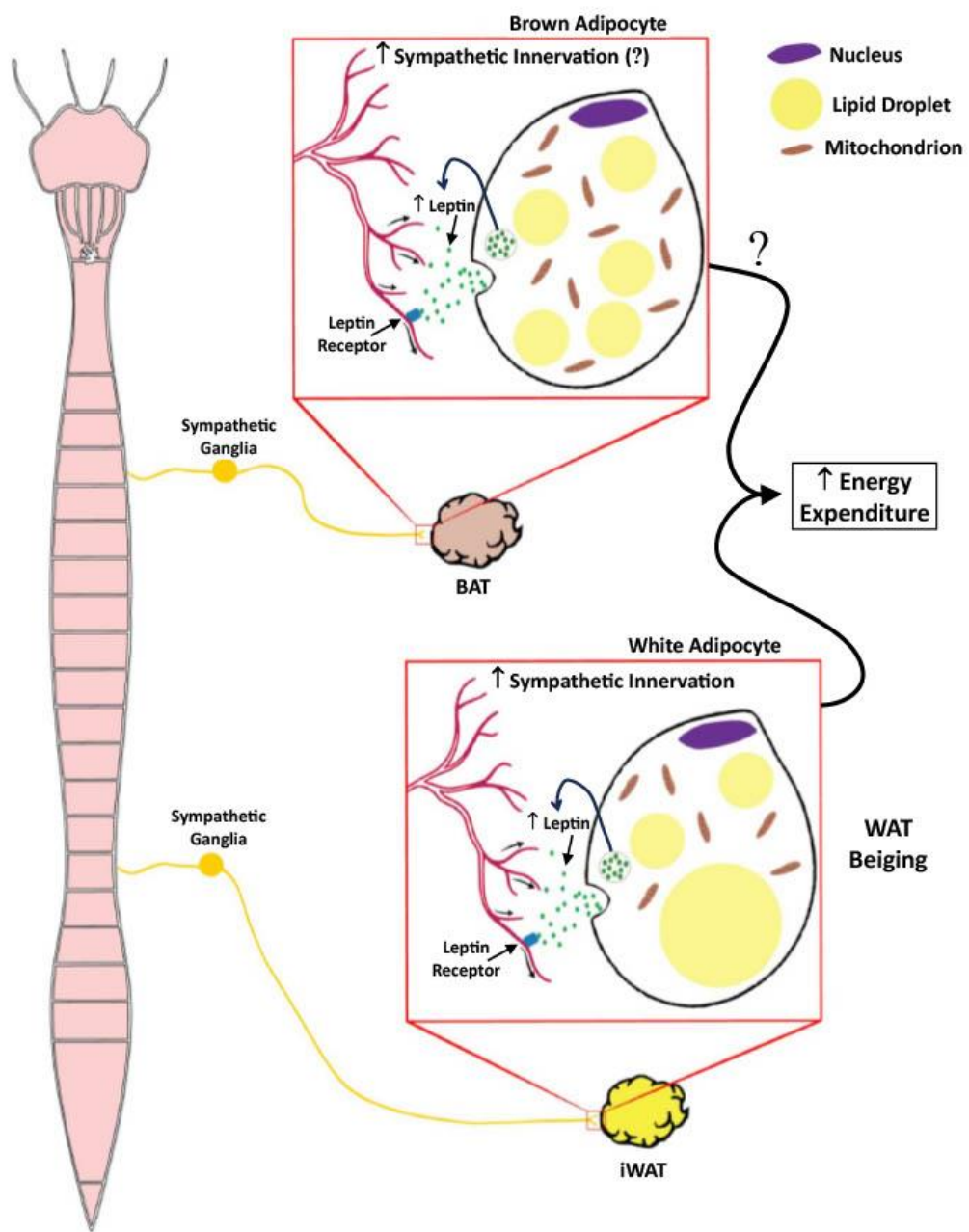
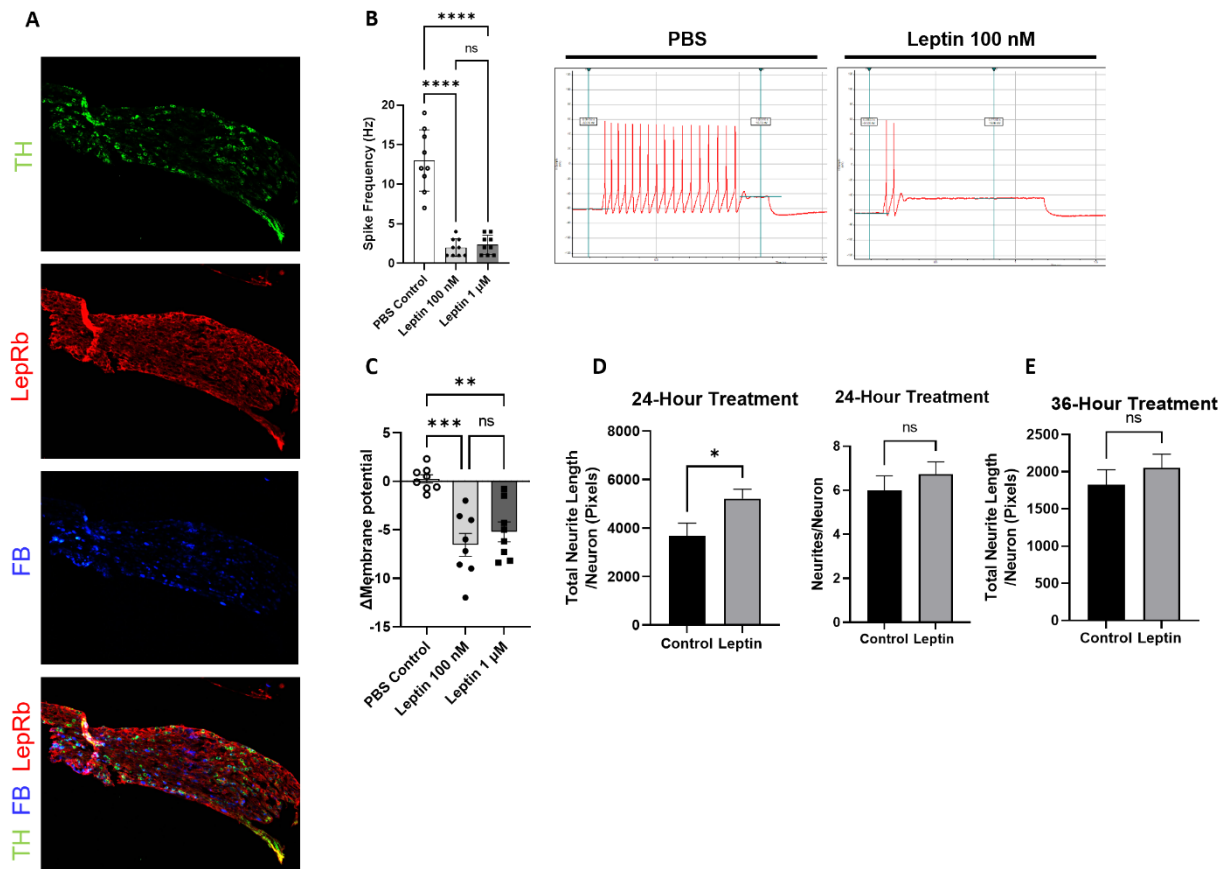
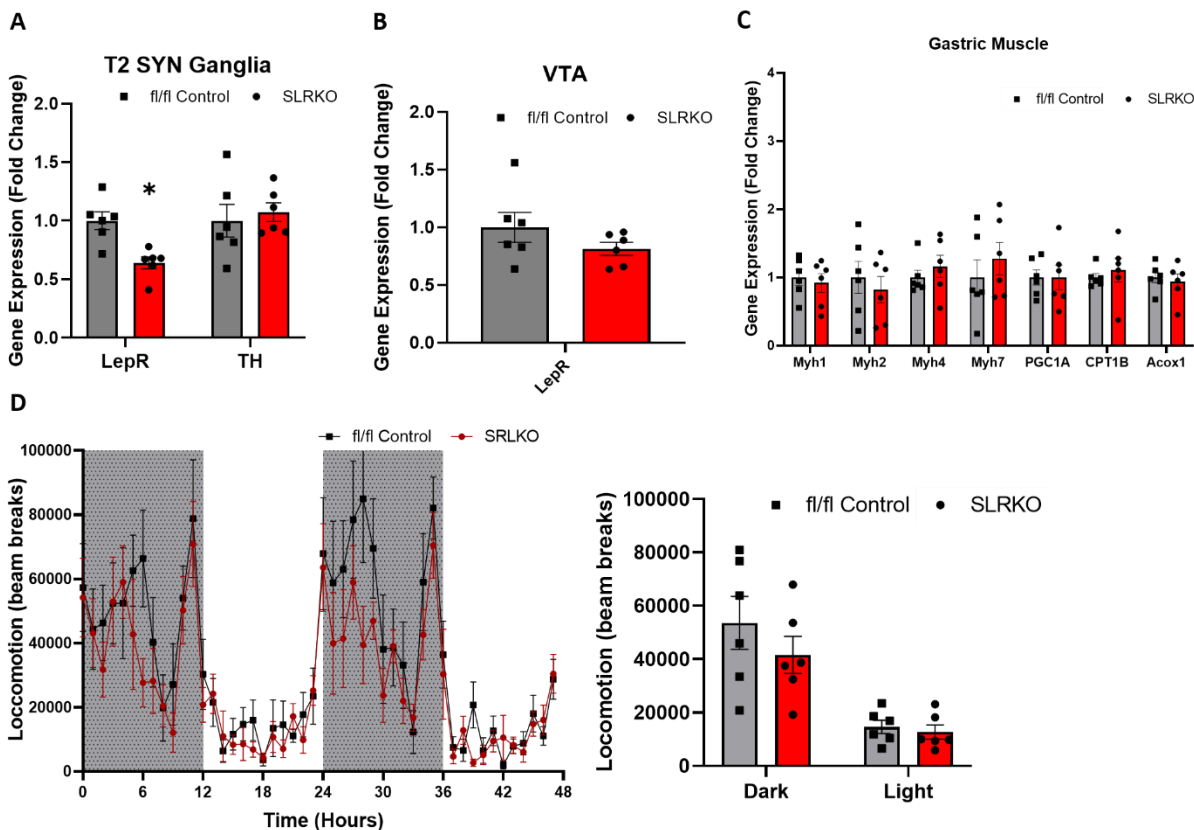


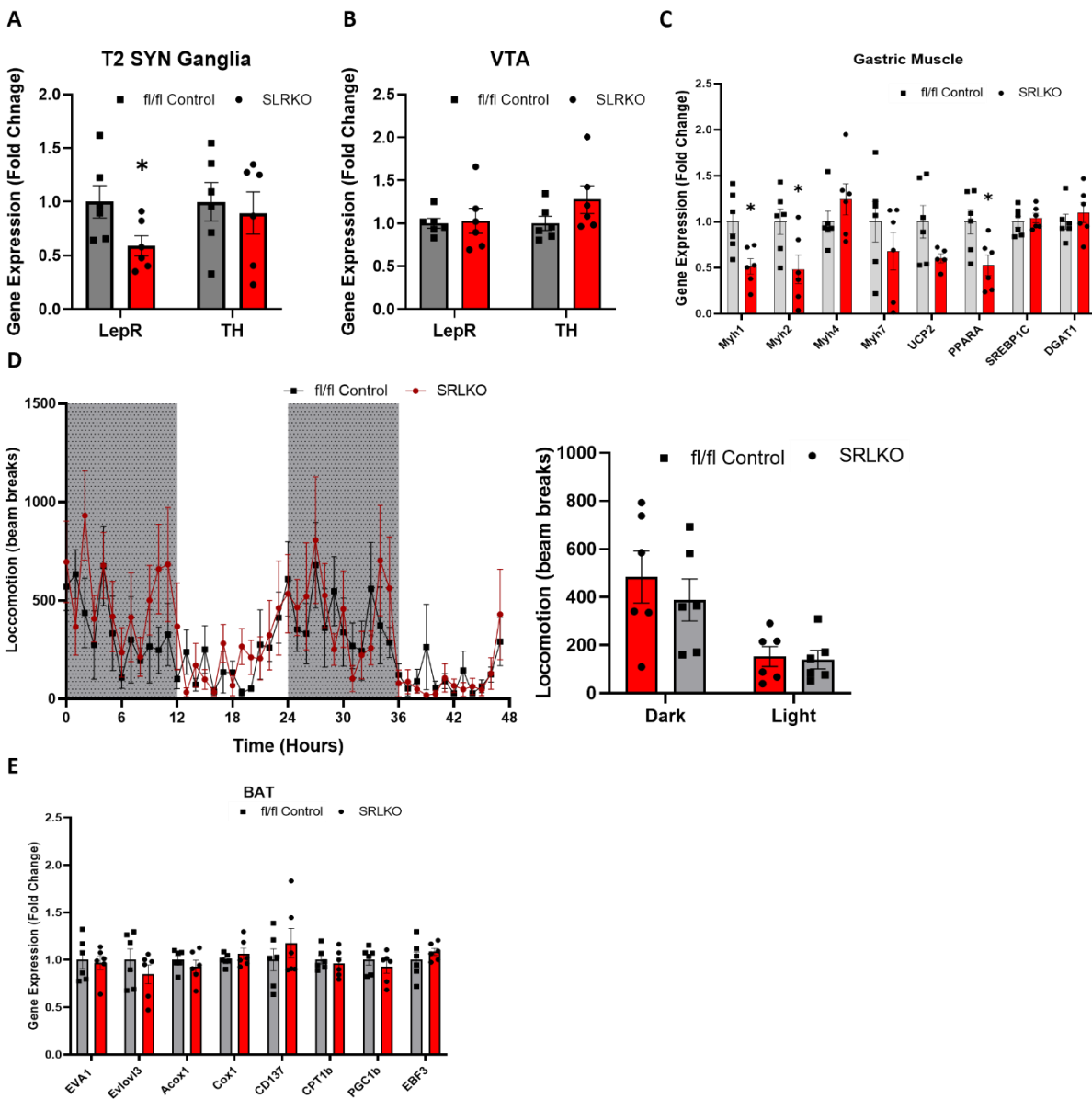
Figure 12. Schematic illustration of the role of adipose tissue-derived leptin and LepRb signaling in the regulation of SNS innervation of adipose tissue. Leptin is secreted from adipose tissue acts through its receptor, LepRb, in sympathetic neurons to promote SNS innervation of iWAT and potentially iBAT.



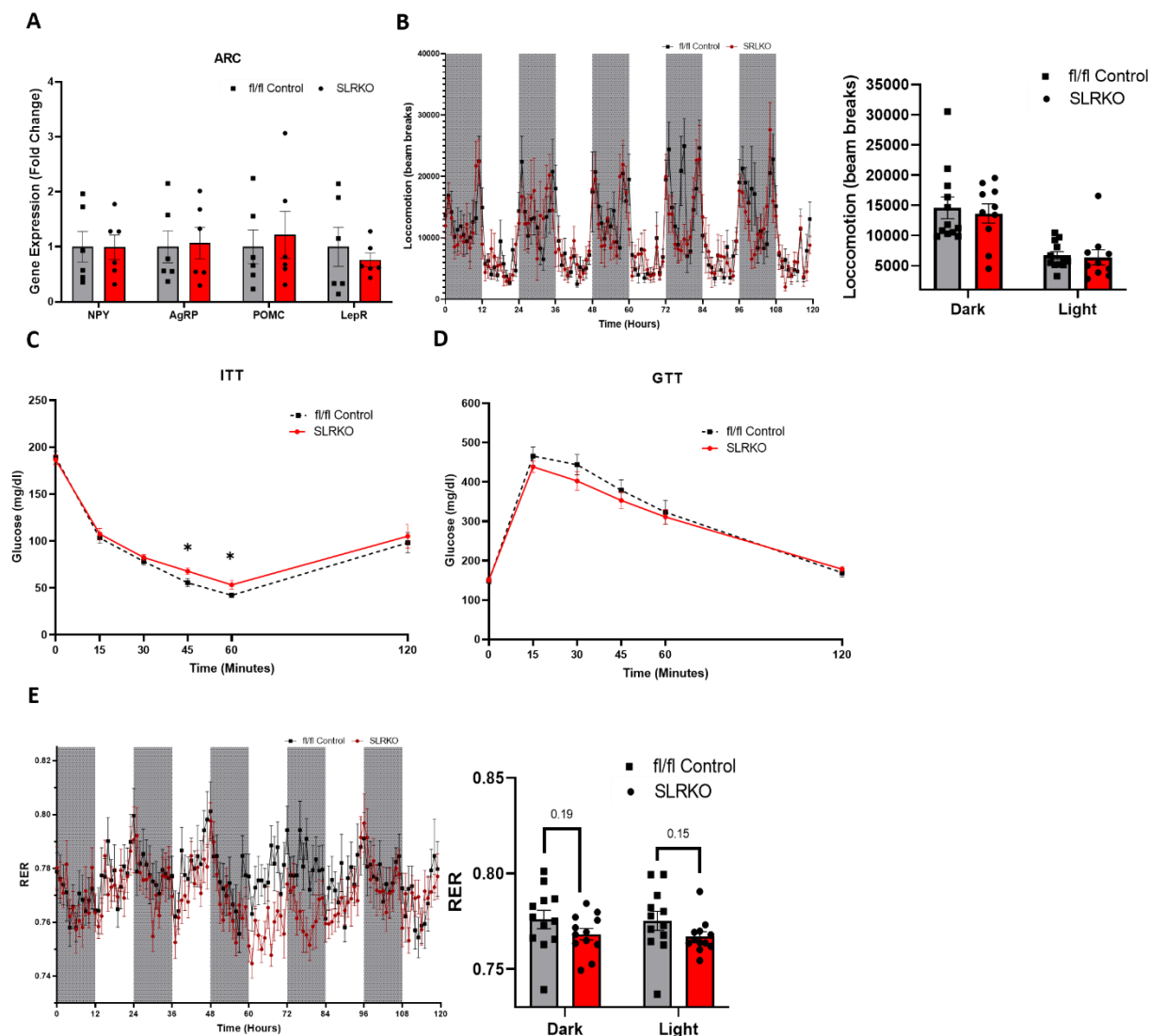
*Supplementary Figure 1. LepR is expressed in T2 sympathetic ganglia and Leptin regulates sympathetic neuron membrane potential and activity. (A) Representative images of TH, Fast Blue (FB) and LepRb-TdTomato labeling in T2 sympathetic ganglia. (B) Spike Frequency of sympathetic neurons in response to a 20 pA depolarizing current ($n = 9$ for all groups). (C) Change in membrane potential of sympathetic neurons after PBS or Leptin treatments ($n = 8$ for all groups). (D) Quantitation of the number of neurites per neuron, total neurite length per neuron of sympathetic ganglia neurons treated with Leptin (100 ng/ml for 24 hours) or PBS from 6-week-old LepR-Cre::Ai14 reporter mice (PBS $n = 16$, Leptin $n = 18$). (E) Quantitation of the total neurite length per neuron of sympathetic ganglia neurons treated with Leptin (100 ng/ml for 36 hours) or PBS from 6-week-old LepR-Cre::Ai14 reporter mice (PBS $n = 13$, Leptin $n = 9$). All data are expressed as mean \pm SEM; * $p < 0.05$.*



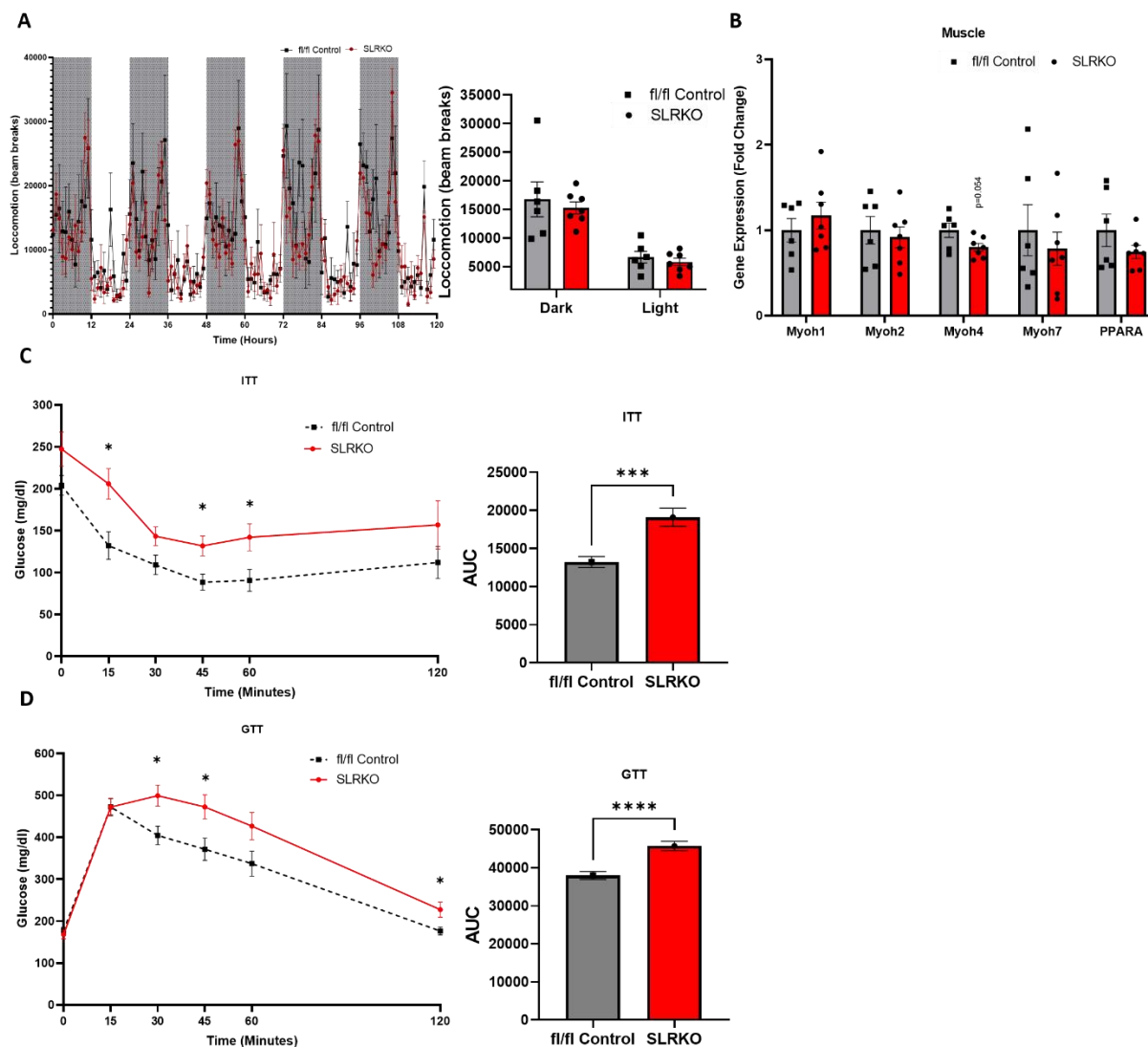
Supplementary Figure 2. *LepR* deficiency in sympathetic neurons of 6-week-old female mice does not affect locomotor activity and *LepR* mRNA levels in the VTA. (A) KO efficiency of SLRKO female mice ($n = 6$). (B) *LepR* mRNA levels in the VTA of SLRKO female mice ($n = 6$). (C) Quantitative RT-PCR analysis of muscle fiber and thermogenic genes in gastric muscle ($n = 6$). (D) Locomotor activity measured as beam breaks ($n = 6$; Light phase represented by white background; Dark phase represented by grey background). All data are expressed as mean \pm SEM; * $p < 0.05$.



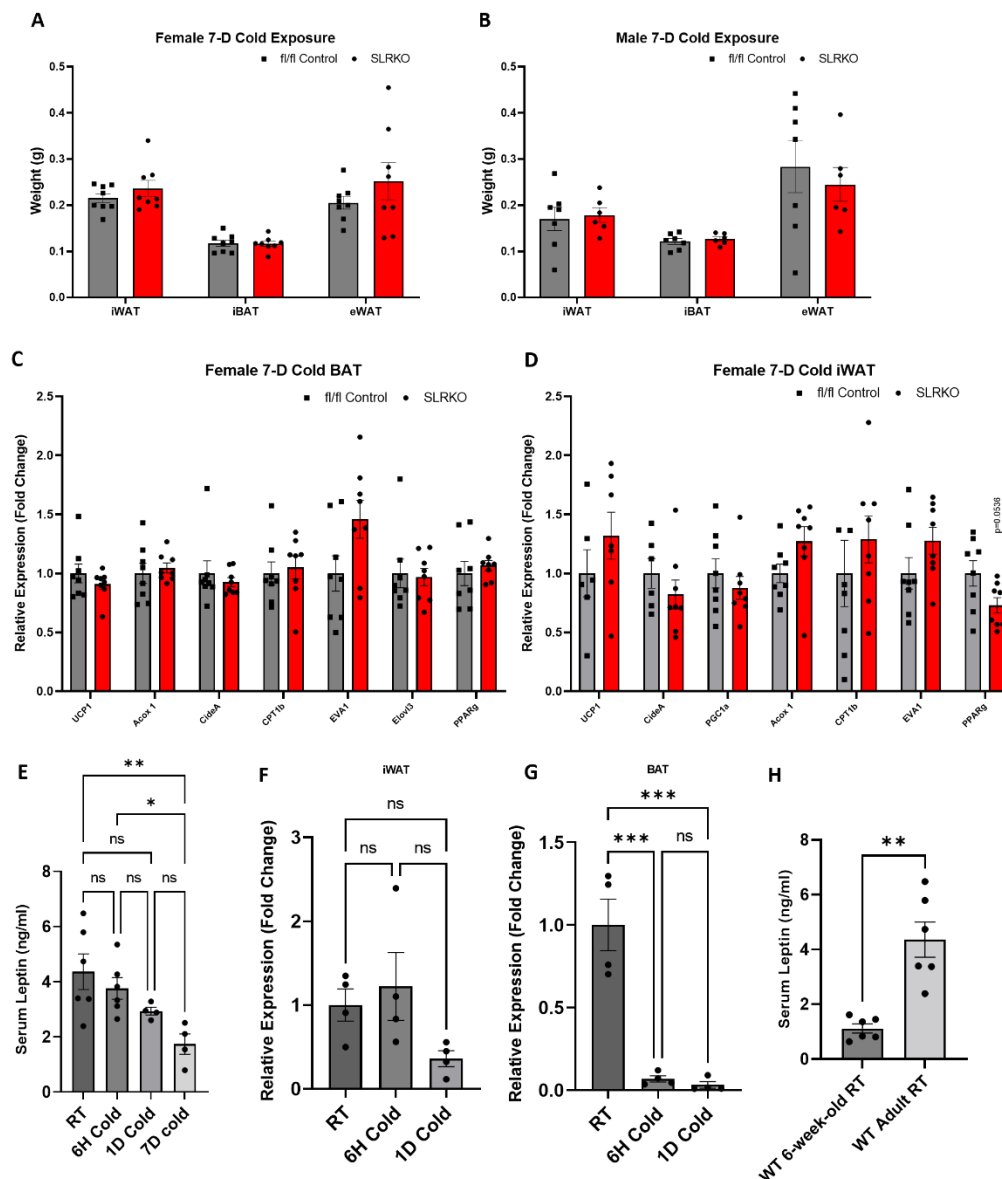
Supplementary Figure 3. *LepR* deficiency in sympathetic neurons of 6-week-old male mice does not affect locomotor activity and *LepR* mRNA levels in the VTA. (A) KO efficiency of SLRKO female mice ($n = 6$). (B) *LepR* mRNA levels in the VTA of SLRKO female mice ($n = 6$). (C) Quantitative RT-PCR analysis of muscle fiber and thermogenic genes in gastric muscle ($n = 6$). (D) Locomotor activity in measured as beam breaks ($n = 6$; Light phase represented by white background; Dark phase represented by grey background). (E) Quantitative RT-PCR analysis of additional thermogenic genes in iBAT ($n = 5-6$). All data are expressed as mean \pm SEM; * $p < 0.05$.



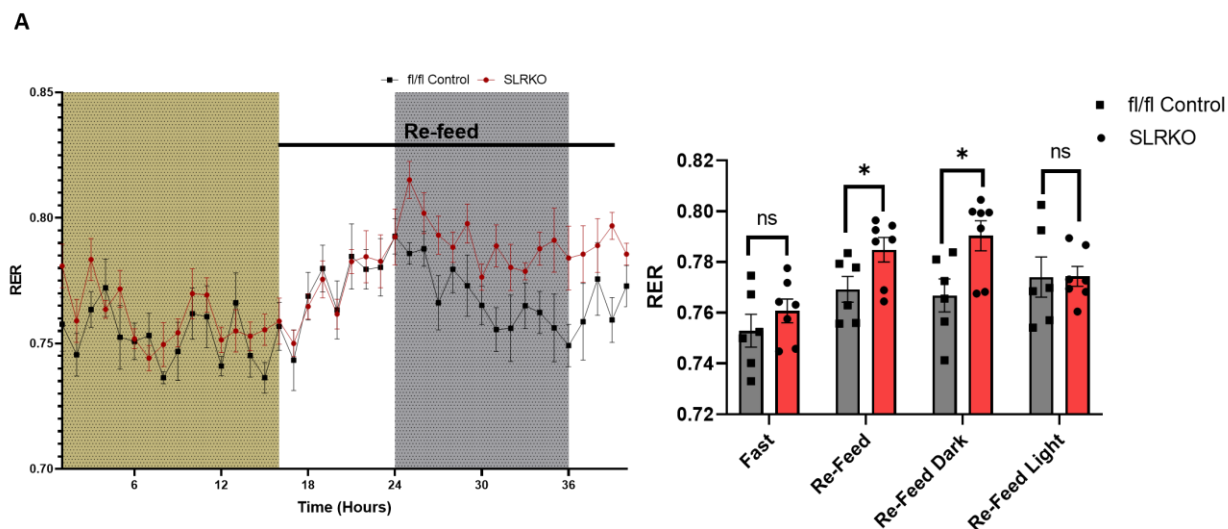
Supplementary Figure 4. HFD-fed female mice with sympathetic neuron specific *LepR* deletion exhibit no differences in ARC neuropeptide and *LepR* expression, locomotor activity, and mild differences in glucose homeostasis. (A) Quantitative RT-PCR analysis of ARC neuropeptides and *LepR* ($n = 6$). (B) Locomotor activity measured as beam breaks ($n = 10-11$; Light phase represented by white background; Dark phase represented by grey background). (C) Insulin tolerance test in female mice on HFD for 12 weeks, measurements recorded during the animal's 18th week of life ($n = 12$ for both groups). (D) Glucose tolerance test in female mice on HFD for 14 weeks, measurements recorded during the animal's 20th week of life ($n = 12$ for both groups). (E) Respiratory exchange ratio ($n = 12$ for both groups; Light phase represented by white background; Dark phase represented by grey background). All data are expressed as mean \pm SEM; * $p < 0.05$.



Supplementary Figure 5. HFD-fed male mice with sympathetic neuron specific *LepR* deletion exhibit defects in glucose homeostasis. (A) Locomotor activity measured as beam breaks ($n = 6-7$; Light phase represented by white background; Dark phase represented by grey background). (B) Quantitative RT-PCR analysis of muscle fiber mRNA expression in gastric muscle ($n = 6$; Light phase represented by white background; Dark phase represented by grey background). (C) Insulin tolerance test in male mice on HFD for 13 weeks, measurements recorded during the animal's 19th week of life (*fl/fl* $n = 10$, SLRKO $n = 11$). (D) Glucose tolerance test in male mice on HFD for 15 weeks, measurements recorded during the animal's 21st week of life (*fl/fl* $n = 10$, SLRKO $n = 11$). All data are expressed as mean \pm SEM; * $p < 0.05$.



Supplementary Figure 6. Adult female and male mice with sympathetic neuron specific *LepR* deletion do not exhibit defects in cold-induced thermogenesis. 6-month-old female and male SLRKO and *fl/fl* littermates were placed in a 7-day cold challenge (4°C). (A) Female fat pad weight ($n = 8$ for both groups). (B) Male fat pad weight (*fl/fl* $n = 7$, SLRKO $n = 6$). (C) Quantitative RT-PCR analysis of thermogenic gene expression in iBAT for female mice ($n = 8$ for both groups). (D) Quantitative RT-PCR analysis of thermogenic gene expression in iWAT for female mice ($n = 8$ for both groups). (E-G) 6-month-old WT mice were cold challenged at 4°C with varying lengths (6-hours, 1-day, 7-days) or kept in room temperature (RT). Following each cold challenge, blood, iWAT, and iBAT tissues were isolated and used to measure, serum leptin content (RT & 6H cold $n = 6$, 1D cold & 7D cold $n = 4$), iWAT Leptin mRNA expression ($n = 4$), and iBAT Leptin mRNA expression ($n = 4$). (H) Serum leptin protein content ($n = 6$ for both groups). All data are expressed as mean \pm SEM; * $p < 0.05$.



Supplementary Figure 7. HFD-fed female mice with sympathetic neuron specific *LepR* deletion exhibit impaired diet-induced thermogenesis. HFD-fed female SLRKO and fl/fl littermates were placed in thermoneutral conditions (30 °C) then fasted for 16 hours (represented by the gold background) and re-fed for 24 hours (light phase represented by white background; dark phase represented by the grey background). (A) Respiratory exchange rate ($n = 6-7$). All data are expressed as mean \pm SEM; * $p < 0.05$.

Antibody	Company	Catalog #	Application
UCP1	Abcam	ab23841	WB (1:1000)
UCP1	Abcam	Ab10983	IHC (1:500)
TH	Millipore	Ab152	WB (1:1000), IF (1:500, 1:1000 for whole mount clearing)
TH	Abcam	Ab76442	IF (1:1000)
β III-tubulin, Alexa Fluor® 488 Conjugate	Millipore	AB15708A4	IF (1:400)
α -Tubulin	Advanced BioChemicals	ABCENT4777	WB (1:1000)
Biotin-SP (long spacer) AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	711-065-152	IHC (1:500)
Cy™3 AffiniPure Donkey Anti- Rabbit IgG (H+L)	Jackson ImmunoResearch	711-165-152	IF (1:500, 1:2000 for whole mount clearing)
Alexa Fluor® 488 AffiniPure Donkey Anti-Chicken IgY (IgG) (H+L)	Jackson ImmunoResearch	703-545-155	IF (1:500)
Donkey anti-Goat IgG (H+L) Cross- Adsorbed Secondary Antibody, Alexa Fluor 680	Invitrogen	A21084	WB (1: 5000)
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 680	Invitrogen	A21109	WB (1:5000)

Supplemental Table 1: Antibodies used in immunoblotting.

7 REFERENCES

- Ahima, R. S., Bjorbaek, C., Osei, S., & Flier, J. S. (1999). Regulation of neuronal and glial proteins by leptin: implications for brain development. *Endocrinology*, *140*(6), 2755–2762.
<https://doi.org/10.1210/endo.140.6.6774>
- Albert, V., Svensson, K., Shimobayashi, M., Colombi, M., Muñoz, S., Jimenez, V., Handschin, C., Bosch, F., & Hall, M. N. (2016). mTORC2 sustains thermogenesis via Akt-induced glucose uptake and glycolysis in brown adipose tissue. *EMBO molecular medicine*, *8*(3), 232–246.
<https://doi.org/10.15252/emmm.201505610>
- Altar, C. A., Cai, N., Bliven, T., Juhasz, M., Conner, J. M., Acheson, A. L., Lindsay, R. M., & Wiegand, S. J. (1997). Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature*, *389*(6653), 856–860. <https://doi.org/10.1038/39885>
- An, J. J., Liao, G. Y., Kinney, C. E., Sahibzada, N., & Xu, B. (2015). Discrete BDNF Neurons in the Paraventricular Hypothalamus Control Feeding and Energy Expenditure. *Cell metabolism*, *22*(1), 175–188. <https://doi.org/10.1016/j.cmet.2015.05.008>
- Bachman, E. S., Dhillon, H., Zhang, C. Y., Cinti, S., Bianco, A. C., Kobilka, B. K., & Lowell, B. B. (2002). betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science (New York, N.Y.)*, *297*(5582), 843–845. <https://doi.org/10.1126/science.1073160>
- Badoer E. (2001). Hypothalamic paraventricular nucleus and cardiovascular regulation. *Clinical and experimental pharmacology & physiology*, *28*(1-2), 95–99. <https://doi.org/10.1046/j.1440-1681.2001.03413.x>
- Bains, J. S., & Ferguson, A. V. (1995). Paraventricular nucleus neurons projecting to the spinal cord receive excitatory input from the subfornical organ. *The American journal of physiology*, *268*(3 Pt 2), R625–R633. <https://doi.org/10.1152/ajpregu.1995.268.3.R625>

- Balland, E., Dam, J., Langlet, F., Caron, E., Steculorum, S., Messina, A., Rasika, S., Falluel-Morel, A., Anouar, Y., Dehouck, B., Trinquet, E., Jockers, R., Bouret, S. G., & Prévot, V. (2014). Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. *Cell metabolism*, *19*(2), 293–301. <https://doi.org/10.1016/j.cmet.2013.12.015>
- Bal, N. C., Singh, S., Reis, F. C. G., Maurya, S. K., Pani, S., Rowland, L. A., & Periasamy, M. (2017). Both brown adipose tissue and skeletal muscle thermogenesis processes are activated during mild to severe cold adaptation in mice. *The Journal of biological chemistry*, *292*(40), 16616–16625. <https://doi.org/10.1074/jbc.M117.790451>
- Balthasar, N., Coppari, R., McMinn, J., Liu, S. M., Lee, C. E., Tang, V., Kenny, C. D., McGovern, R. A., Chua, S. C., Jr, Elmquist, J. K., & Lowell, B. B. (2004). Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron*, *42*(6), 983–991. <https://doi.org/10.1016/j.neuron.2004.06.004>
- Barbatelli, G., Murano, I., Madsen, L., Hao, Q., Jimenez, M., Kristiansen, K., Giacobino, J. P., De Matteis, R., & Cinti, S. (2010). The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *American journal of physiology. Endocrinology and metabolism*, *298*(6), E1244–E1253. <https://doi.org/10.1152/ajpendo.00600.2009>
- Bareyre, F. M., Garzorz, N., Lang, C., Misgeld, T., Büning, H., & Kerschensteiner, M. (2011). In vivo imaging reveals a phase-specific role of STAT3 during central and peripheral nervous system axon regeneration. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(15), 6282–6287. <https://doi.org/10.1073/pnas.1015239108>
- Bartelt, A., Bruns, O. T., Reimer, R., Hohenberg, H., Ittrich, H., Peldschus, K., Kaul, M. G., Tromsdorf, U. I., Weller, H., Waurisch, C., Eychmüller, A., Gordts, P. L., Rinninger, F., Bruegelmann, K., Freund, B., Nielsen, P., Merkel, M., & Heeren, J. (2011). Brown adipose tissue activity controls triglyceride clearance. *Nature medicine*, *17*(2), 200–205. <https://doi.org/10.1038/nm.2297>

- Bartness, T. J., Liu, Y., Shrestha, Y. B., & Ryu, V. (2014). Neural innervation of white adipose tissue and the control of lipolysis. *Frontiers in neuroendocrinology*, 35(4), 473–493.
<https://doi.org/10.1016/j.yfrne.2014.04.001>
- Bartness, T. J., Shrestha, Y. B., Vaughan, C. H., Schwartz, G. J., & Song, C. K. (2010). Sensory and sympathetic nervous system control of white adipose tissue lipolysis. *Molecular and cellular endocrinology*, 318(1-2), 34–43. <https://doi.org/10.1016/j.mce.2009.08.031>
- Bartness, T. J., & Song, C. K. (2007). Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue. *Journal of lipid research*, 48(8), 1655–1672.
<https://doi.org/10.1194/jlr.R700006-JLR200>
- Bartness, T. J., & Wade, G. N. (1984). Effects of interscapular brown adipose tissue denervation on body weight and energy metabolism in ovariectomized and estradiol-treated rats. *Behavioral neuroscience*, 98(4), 674–685. <https://doi.org/10.1037//0735-7044.98.4.674>
- Bastías-Pérez, M., Zagmutt, S., Soler-Vázquez, M. C., Serra, D., Mera, P., & Herrero, L. (2020). Impact of Adaptive Thermogenesis in Mice on the Treatment of Obesity. *Cells*, 9(2), 316.
<https://doi.org/10.3390/cells9020316>
- Baumann, H., Morella, K. K., White, D. W., Dembski, M., Bailon, P. S., Kim, H., Lai, C. F., & Tartaglia, L. A. (1996). The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proceedings of the National Academy of Sciences of the United States of America*, 93(16), 8374–8378. <https://doi.org/10.1073/pnas.93.16.8374>
- Begg, G. E., Carrington, L., Stokes, P. H., Matthews, J. M., Wouters, M. A., Husain, A., Lorand, L., Iismaa, S. E., & Graham, R. M. (2006). Mechanism of allosteric regulation of Transglutaminase 2 by GTP. *Proceedings of the National Academy of Sciences*, 103(52), 19683–19688.
<https://doi.org/10.1073/pnas.0609283103>
- Bereiter, D. A., & Jeanrenaud, B. (1980). Altered dendritic orientation of hypothalamic neurons from genetically obese (ob/ob) mice. *Brain research*, 202(1), 201–206.

- Bereiter, D. A., & Jeanrenaud, B. (1979). Altered neuroanatomical organization in the central nervous system of the genetically obese (ob/ob) mouse. *Brain research*, *165*(2), 249–260.
[https://doi.org/10.1016/0006-8993\(79\)90557-2](https://doi.org/10.1016/0006-8993(79)90557-2)
- Bernhard, F., Landgraf, K., Klöting, N., Berthold, A., Büttner, P., Friebe, D., Kiess, W., Kovacs, P., Blüher, M., & Körner, A. (2013). Functional relevance of genes implicated by obesity genome-wide association study signals for human adipocyte biology. *Diabetologia*, *56*(2), 311–322.
<https://doi.org/10.1007/s00125-012-2773-0>
- Bertholet, A. M., Kazak, L., Chouchani, E. T., Bogaczyńska, M. G., Paranjpe, I., Wainwright, G. L., Bétourné, A., Kajimura, S., Spiegelman, B. M., & Kirichok, Y. (2017). Mitochondrial Patch Clamp of Beige Adipocytes Reveals UCP1-Positive and UCP1-Negative Cells Both Exhibiting Futile Creatine Cycling. *Cell metabolism*, *25*(4), 811–822.e4.
<https://doi.org/10.1016/j.cmet.2017.03.002>
- Billington, C. J., Briggs, J. E., Harker, S., Grace, M., & Levine, A. S. (1994). Neuropeptide Y in hypothalamic paraventricular nucleus: a center coordinating energy metabolism. *The American journal of physiology*, *266*(6 Pt 2), R1765–R1770.
<https://doi.org/10.1152/ajpregu.1994.266.6.R1765>
- Bingham, N. C., Anderson, K. K., Reuter, A. L., Stallings, N. R., & Parker, K. L. (2008). Selective loss of leptin receptors in the ventromedial hypothalamic nucleus results in increased adiposity and a metabolic syndrome. *Endocrinology*, *149*(5), 2138–2148. <https://doi.org/10.1210/en.2007-1200>
- Blaszkiwicz, M., Wood, E., Koizar, S., Willows, J., Anderson, R., Tseng, Y. H., Godwin, J., & Townsend, K. L. (2020). The involvement of neuroimmune cells in adipose innervation. *Molecular medicine (Cambridge, Mass.)*, *26*(1), 126. [https://doi.org/10.1186/s10020-020-00254-](https://doi.org/10.1186/s10020-020-00254-3)

- Bouret, S. G., Draper, S. J., & Simerly, R. B. (2004). Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science (New York, N.Y.)*, *304*(5667), 108–110.
<https://doi.org/10.1126/science.1095004>
- Bouret, S. G., & Simerly, R. B. (2007). Development of leptin-sensitive circuits. *Journal of neuroendocrinology*, *19*(8), 575–582. <https://doi.org/10.1111/j.1365-2826.2007.01563.x>
- Bowers, R. R., Festuccia, W. T., Song, C. K., Shi, H., Migliorini, R. H., & Bartness, T. J. (2004). Sympathetic innervation of white adipose tissue and its regulation of fat cell number. *American journal of physiology. Regulatory, integrative and comparative physiology*, *286*(6), R1167–R1175. <https://doi.org/10.1152/ajpregu.00558.2003>
- Bové, M., Monto, F., Guillem-Llobat, P., Ivorra, M. D., Noguera, M. A., Zambrano, A., Sirerol-Piquer, M. S., Requena, A. C., García-Alonso, M., Tejerina, T., Real, J. T., Fariñas, I., & D'Ocon, P. (2021). NT3/TrkC Pathway Modulates the Expression of UCP-1 and Adipocyte Size in Human and Rodent Adipose Tissue. *Frontiers in endocrinology*, *12*, 630097.
<https://doi.org/10.3389/fendo.2021.630097>
- Bradley, R. L., Mansfield, J. P., & Maratos-Flier, E. (2005). Neuropeptides, including neuropeptide Y and melanocortins, mediate lipolysis in murine adipocytes. *Obesity research*, *13*(4), 653–661.
<https://doi.org/10.1038/oby.2005.73>
- Caballero, B. (2019). Humans against Obesity: Who Will Win?. *Advances in nutrition (Bethesda, Md.)*, *10*(suppl_1), S4–S9. <https://doi.org/10.1093/advances/nmy055>
- Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R., & Burn, P. (1995). Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science (New York, N.Y.)*, *269*(5223), 546–549. <https://doi.org/10.1126/science.7624778>
- Cannon, B., & Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiological reviews*, *84*(1), 277–359. <https://doi.org/10.1152/physrev.00015.2003>

- Cantu, R. C., & Goodman, H. M. (1967). Effects of denervation and fasting on white adipose tissue. *The American journal of physiology*, 212(1), 207–212.
<https://doi.org/10.1152/ajplegacy.1967.212.1.207>
- Cao, Q., Jing, J., Cui, X., Shi, H., & Xue, B. (2019). Sympathetic nerve innervation is required for beigeing in white fat. *Physiological reports*, 7(6), e14031. <https://doi.org/10.14814/phy2.14031>
- Caron, A., Lee, S., Elmquist, J. K., & Gautron, L. (2018). Leptin and brain–adipose crosstalks. *Nature Reviews Neuroscience*, 19(3), 153–165. <https://doi.org/10.1038/nrn.2018.7>
- Chamberlain, K. A., & Sheng, Z. H. (2019). Mechanisms for the maintenance and regulation of axonal energy supply. *Journal of neuroscience research*, 97(8), 897–913.
<https://doi.org/10.1002/jnr.24411>
- Chen, H., & Chan, D. C. (2009). Mitochondrial dynamics—fusion, fission, movement, and mitophagy—in neurodegenerative diseases. *Human molecular genetics*, 18(R2), R169–R176.
<https://doi.org/10.1093/hmg/ddp326>
- Chen, W. V., & Maniatis, T. (2013). Clustered protocadherins. *Development (Cambridge, England)*, 140(16), 3297–3302. <https://doi.org/10.1242/dev.090621>
- Chen, W. V., Nwakeze, C. L., Denny, C. A., O'Keeffe, S., Rieger, M. A., Mountoufaris, G., Kirner, A., Dougherty, J. D., Hen, R., Wu, Q., & Maniatis, T. (2017). *Pcdhac2* is required for axonal tiling and assembly of serotonergic circuitries in mice. *Science (New York, N.Y.)*, 356(6336), 406–411.
<https://doi.org/10.1126/science.aal3231>
- Chi, J., Lin, Z., Barr, W., Crane, A., Zhu, X. G., & Cohen, P. (2021). Early postnatal interactions between beige adipocytes and sympathetic neurites regulate innervation of subcutaneous fat. *eLife*, 10, e64693. <https://doi.org/10.7554/eLife.64693>
- Chi, J., Crane, A., Wu, Z., & Cohen, P. (2018). Adipo-Clear: A Tissue Clearing Method for Three-Dimensional Imaging of Adipose Tissue. *Journal of visualized experiments : JoVE*, (137), 58271.
<https://doi.org/10.3791/58271>

- Chi, J., Wu, Z., Choi, C. H. J., Nguyen, L., Teegene, S., Ackerman, S. E., Crane, A., Marchildon, F., Tessier-Lavigne, M., & Cohen, P. (2018). Three-Dimensional Adipose Tissue Imaging Reveals Regional Variation in Beige Fat Biogenesis and PRDM16-Dependent Sympathetic Neurite Density. *Cell metabolism*, 27(1), 226–236.e3. <https://doi.org/10.1016/j.cmet.2017.12.011>
- Choo, M., Miyazaki, T., Yamazaki, M., Kawamura, M., Nakazawa, T., Zhang, J., Tanimura, A., Uesaka, N., Watanabe, M., Sakimura, K., & Kano, M. (2017). Retrograde BDNF to TrkB signaling promotes synapse elimination in the developing cerebellum. *Nature communications*, 8(1), 195. <https://doi.org/10.1038/s41467-017-00260-w>
- Christoffolete, M. A., Linardi, C. C. G., De Jesus, L., Ebina, K. N., Carvalho, S. D., Ribeiro, M. O., Rabelo, R., Curcio, C., Martins, L., Kimura, E. T., & Bianco, A. C. (2004). Mice with Targeted Disruption of the Dio2 Gene Have Cold-Induced Overexpression of the Uncoupling Protein 1 Gene but Fail to Increase Brown Adipose Tissue Lipogenesis and Adaptive Thermogenesis. *Diabetes*, 53 (3): 577–584. <https://doi.org/10.2337/diabetes.53.3.577>
- Cohen, P., Zhao, C., Cai, X., Montez, J. M., Rohani, S. C., Feinstein, P., Mombaerts, P., & Friedman, J. M. (2001). Selective deletion of leptin receptor in neurons leads to obesity. *The Journal of clinical investigation*, 108(8), 1113–1121. <https://doi.org/10.1172/JCI13914>
- Collins S, Kuhn CM, Petro AE, Swick AG, Chrnyk BA, Surwit RS. (1996) Role of leptin in fat regulation. *Nature*.;380:677. <https://doi.org/10.1038/380677a0>
- Correll J. W. (1963). Adipose tissue: ability to respond to nerve stimulation in vitro. *Science (New York, N.Y.)*, 140(3565), 387–388. <https://doi.org/10.1126/science.140.3565.387>
- Côté, I., Sakarya, Y., Green, S. M., Morgan, D., Carter, C. S., Tümer, N., & Scarpace, P. J. (2018). iBAT sympathetic innervation is not required for body weight loss induced by central leptin delivery. *American journal of physiology. Endocrinology and metabolism*, 314(3), E224–E231. <https://doi.org/10.1152/ajpendo.00219.2017>

- Cowley, M. A., Smart, J. L., Rubinstein, M., Cerdán, M. G., Diano, S., Horvath, T. L., Cone, R. D., & Low, M. J. (2001). Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*, *411*(6836), 480–484. <https://doi.org/10.1038/35078085>
- Cui, X., Jing, J., Wu, R., Cao, Q., Li, F., Li, K., Wang, S., Yu, L., Schwartz, G., Shi, H., Xue, B., & Shi, H. (2021). Adipose tissue-derived neurotrophic factor 3 regulates sympathetic innervation and thermogenesis in adipose tissue. *Nature communications*, *12*(1), 5362. <https://doi.org/10.1038/s41467-021-25766-2>
- Cui, X., Nguyen, N. L., Zarebidaki, E., Cao, Q., Li, F., Zha, L., Bartness, T., Shi, H., & Xue, B. (2016). Thermoneutrality decreases thermogenic program and promotes adiposity in high-fat diet-fed mice. *Physiological reports*, *4*(10), e12799. <https://doi.org/10.14814/phy2.12799>
- Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., & Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *The New England journal of medicine*, *360*(15), 1509–1517. <https://doi.org/10.1056/NEJMoa0810780>
- Czaja, K., Lakomy, M., Kaleczyc, J., Barb, C. R., Rampacek, G. B., & Kraeling, R. R. (2002). Leptin receptors, NPY, and tyrosine hydroxylase in autonomic neurons supplying fat depots in a pig. *Biochemical and biophysical research communications*, *293*(3), 1138–1144. [https://doi.org/10.1016/S0006-291X\(02\)00335-2](https://doi.org/10.1016/S0006-291X(02)00335-2)
- DeFalco, J., Tomishima, M., Liu, H., Zhao, C., Cai, X., Marth, J. D., Enquist, L., & Friedman, J. M. (2001). Virus-assisted mapping of neural inputs to a feeding center in the hypothalamus. *Science (New York, N.Y.)*, *291*(5513), 2608–2613. <https://doi.org/10.1126/science.1056602>
- Delghandi, M. P., Johannessen, M., & Moens, U. (2005). The cAMP signalling pathway activates CREB through PKA, p38 and MSK1 in NIH 3T3 cells. *Cellular signalling*, *17*(11), 1343–1351. <https://doi.org/10.1016/j.cellsig.2005.02.003>
- Demas, G. E., & Bartness, T. J. (2001). Direct innervation of white fat and adrenal medullary catecholamines mediate photoperiodic changes in body fat. *American journal of physiology*.

Regulatory, integrative and comparative physiology, 281(5), R1499–R1505.

<https://doi.org/10.1152/ajpregu.2001.281.5.R1499>

Dent, E. W., Gupton, S. L., & Gertler, F. B. (2011). The growth cone cytoskeleton in axon outgrowth and guidance. *Cold Spring Harbor perspectives in biology*, 3(3), a001800.

<https://doi.org/10.1101/cshperspect.a001800>

Dhillon, H., Zigman, J. M., Ye, C., Lee, C. E., McGovern, R. A., Tang, V., Kenny, C. D., Christiansen, L. M., White, R. D., Edelman, E. A., Coppari, R., Balthasar, N., Cowley, M. A., Chua, S., Jr, Elmquist, J. K., & Lowell, B. B. (2006). Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron*, 49(2), 191–203.

<https://doi.org/10.1016/j.neuron.2005.12.021>

Dib, B., Rompré, P. P., Amir, S., & Shizgal, P. (1994). Thermogenesis in brown adipose tissue is activated by electrical stimulation of the rat dorsal raphe nucleus. *Brain research*, 650(1), 149–152. [https://doi.org/10.1016/0006-8993\(94\)90218-6](https://doi.org/10.1016/0006-8993(94)90218-6)

do Carmo, J. M., da Silva, A. A., Cai, Z., Lin, S., Dubinina, J. H., & Hall, J. E. (2011). Control of blood pressure, appetite, and glucose by leptin in mice lacking leptin receptors in proopiomelanocortin neurons. *Hypertension (Dallas, Tex. : 1979)*, 57(5), 918–926.

<https://doi.org/10.1161/HYPERTENSIONAHA.110.161349>

Dodd, G. T., Worth, A. A., Nunn, N., Korpai, A. K., Bechtold, D. A., Allison, M. B., Myers, M. G., Jr, Statnick, M. A., & Luckman, S. M. (2014). The thermogenic effect of leptin is dependent on a distinct population of prolactin-releasing peptide neurons in the dorsomedial hypothalamus. *Cell metabolism*, 20(4), 639–649. <https://doi.org/10.1016/j.cmet.2014.07.022>

Dong, M., Yang, X., Lim, S., Cao, Z., Honek, J., Lu, H., Zhang, C., Seki, T., Hosaka, K., Wahlberg, E., Yang, J., Zhang, L., Länne, T., Sun, B., Li, X., Liu, Y., Zhang, Y., & Cao, Y. (2013). Cold exposure promotes atherosclerotic plaque growth and instability via UCP1-dependent lipolysis. *Cell metabolism*, 18(1), 118–129. <https://doi.org/10.1016/j.cmet.2013.06.003>

- Dulloo, A. G., & Miller, D. S. (1984). Energy balance following sympathetic denervation of brown adipose tissue. *Canadian journal of physiology and pharmacology*, 62(2), 235–240.
<https://doi.org/10.1139/y84-035>
- Duska, F., Andel, M., Kubena, A., & Macdonald, I. A. (2005). Effects of acute starvation on insulin resistance in obese patients with and without type 2 diabetes mellitus. *Clinical nutrition (Edinburgh, Scotland)*, 24(6), 1056–1064. <https://doi.org/10.1016/j.clnu.2005.08.008>
- Edwards M, Mohiuddin SS. Biochemistry, Lipolysis. [Updated 2023]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from:
<https://www.ncbi.nlm.nih.gov/books/NBK560564/>
- Elias, C. F., Aschkenasi, C., Lee, C., Kelly, J., Ahima, R. S., Bjorbaek, C., Flier, J. S., Saper, C. B., & Elmquist, J. K. (1999). Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron*, 23(4), 775–786. [https://doi.org/10.1016/s0896-6273\(01\)80035-0](https://doi.org/10.1016/s0896-6273(01)80035-0)
- Ellacott, K. L., Morton, G. J., Woods, S. C., Tso, P., & Schwartz, M. W. (2010). Assessment of feeding behavior in laboratory mice. *Cell metabolism*, 12(1), 10–17.
<https://doi.org/10.1016/j.cmet.2010.06.001>
- Elshamy, W. M., & Ernfors, P. (1996). Requirement of neurotrophin-3 for the survival of proliferating trigeminal ganglion progenitor cells. *Development (Cambridge, England)*, 122(8), 2405–2414.
<https://doi.org/10.1242/dev.122.8.2405>
- Enomoto, H., Crawford, P. A., Gorodinsky, A., Heuckeroth, R. O., Johnson, E. M., Jr, & Milbrandt, J. (2001). RET signaling is essential for migration, axonal growth and axon guidance of developing sympathetic neurons. *Development (Cambridge, England)*, 128(20), 3963–3974.
<https://doi.org/10.1242/dev.128.20.3963>
- Enriori, P. J., Sinnayah, P., Simonds, S. E., Garcia Rudaz, C., & Cowley, M. A. (2011). Leptin action in the dorsomedial hypothalamus increases sympathetic tone to brown adipose tissue in spite of

- systemic leptin resistance. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(34), 12189–12197. <https://doi.org/10.1523/JNEUROSCI.2336-11.2011>
- Farzi, A., Lau, J., Ip, C. K., Qi, Y., Shi, Y. C., Zhang, L., Tasan, R., Sperk, G., & Herzog, H. (2018). Arcuate nucleus and lateral hypothalamic CART neurons in the mouse brain exert opposing effects on energy expenditure. *eLife*, 7, e36494. <https://doi.org/10.7554/eLife.36494>
- Fawcett, J. P., Bamji, S. X., Causing, C. G., Aloyz, R., Ase, A. R., Reader, T. A., McLean, J. H., & Miller, F. D. (1998). Functional evidence that BDNF is an anterograde neuronal trophic factor in the CNS. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 18(8), 2808–2821. <https://doi.org/10.1523/JNEUROSCI.18-08-02808.1998>
- Fedorenko, A., Lishko, P. V., & Kirichok, Y. (2012). Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. *Cell*, 151(2), 400–413. <https://doi.org/10.1016/j.cell.2012.09.010>
- Fei, H., Okano, H. J., Li, C., Lee, G. H., Zhao, C., Darnell, R., & Friedman, J. M. (1997). Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proceedings of the National Academy of Sciences of the United States of America*, 94(13), 7001–7005. <https://doi.org/10.1073/pnas.94.13.7001>
- Fernandes, G. W., Ueta, C. B., Fonseca, T. L., Gouveia, C. H., Lancellotti, C. L., Brum, P. C., Christoffolete, M. A., Bianco, A. C., & Ribeiro, M. O. (2014). Inactivation of the adrenergic receptor β 2 disrupts glucose homeostasis in mice. *The Journal of endocrinology*, 221(3), 381–390. <https://doi.org/10.1530/JOE-13-0526>
- Flegal, K. M., Kruszon-Moran, D., Carroll, M. D., Fryar, C. D., & Ogden, C. L. (2016). Trends in Obesity Among Adults in the United States, 2005 to 2014. *JAMA*, 315(21), 2284–2291. <https://doi.org/10.1001/jama.2016.6458>
- Foster, M. T., & Bartness, T. J. (2006). Sympathetic but not sensory denervation stimulates white adipocyte proliferation. *American journal of physiology. Regulatory, integrative and comparative physiology*, 291(6), R1630–R1637. <https://doi.org/10.1152/ajpregu.00197.2006>

- Foster, M. T., Song, C. K., & Bartness, T. J. (2010). Hypothalamic paraventricular nucleus lesion involvement in the sympathetic control of lipid mobilization. *Obesity (Silver Spring, Md.)*, *18*(4), 682–689. <https://doi.org/10.1038/oby.2009.345>
- François, M., Torres, H., Huesing, C., Zhang, R., Saurage, C., Lee, N., Qualls-Creekmore, E., Yu, S., Morrison, C. D., Burk, D., Berthoud, H. R., & Münzberg, H. (2019). Sympathetic innervation of the interscapular brown adipose tissue in mouse. *Annals of the New York Academy of Sciences*, *1454*(1), 3–13. <https://doi.org/10.1111/nyas.14119>
- Friedman J. M. (2019). Leptin and the endocrine control of energy balance. *Nature metabolism*, *1*(8), 754–764. <https://doi.org/10.1038/s42255-019-0095-y>
- Gao, Q., Wolfgang, M. J., Neschen, S., Morino, K., Horvath, T. L., Shulman, G. I., & Fu, X. Y. (2004). Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(13), 4661–4666. <https://doi.org/10.1073/pnas.0303992101>
- Géloën, A., Collet, A. J., & Bukowiecki, L. J. (1992). Role of sympathetic innervation in brown adipocyte proliferation. *The American journal of physiology*, *263*(6 Pt 2), R1176–R1181. <https://doi.org/10.1152/ajpregu.1992.263.6.R1176>
- Glebova, N. O., & Ginty, D. D. (2005). Growth and survival signals controlling sympathetic nervous system development. *Annual review of neuroscience*, *28*, 191–222. <https://doi.org/10.1146/annurev.neuro.28.061604.135659>
- Goldberg J. L. (2003). How does an axon grow?. *Genes & development*, *17*(8), 941–958. <https://doi.org/10.1101/gad.1062303>
- Gómez-García, I., Trepiana, J., Fernández-Quintela, A., Giralt, M., & Portillo, M. P. (2022). Sexual Dimorphism in Brown Adipose Tissue Activation and White Adipose Tissue Browning. *International journal of molecular sciences*, *23*(15), 8250. <https://doi.org/10.3390/ijms23158250>
- Granneman, J. G., Lahners, K. N., & Chaudhry, A. (1991). Molecular cloning and expression of the rat beta 3-adrenergic receptor. *Molecular pharmacology*, *40*(6), 895–899.

- Grujic, D., Susulic, V. S., Harper, M. E., Himms-Hagen, J., Cunningham, B. A., Corkey, B. E., & Lowell, B. B. (1997). Beta3-adrenergic receptors on white and brown adipocytes mediate beta3-selective agonist-induced effects on energy expenditure, insulin secretion, and food intake. A study using transgenic and gene knockout mice. *The Journal of biological chemistry*, 272(28), 17686–17693. <https://doi.org/10.1074/jbc.272.28.17686>
- Gruzdeva, O., Borodkina, D., Uchasova, E., Dyleva, Y., & Barbarash, O. (2019). Leptin resistance: underlying mechanisms and diagnosis. *Diabetes, metabolic syndrome and obesity : targets and therapy*, 12, 191–198. <https://doi.org/10.2147/DMSO.S182406>
- Gundemir, S., Colak, G., Tucholski, J., & Johnson, G. V. (2012). Transglutaminase 2: a molecular Swiss army knife. *Biochimica et biophysica acta*, 1823(2), 406–419. <https://doi.org/10.1016/j.bbamcr.2011.09.012>
- Halaas, J. L., Gajiwala, K. S., Maffei, M., Cohen, S. L., Chait, B. T., Rabinowitz, D., Lallone, R. L., Burley, S. K., & Friedman, J. M. (1995). Weight-reducing effects of the plasma protein encoded by the obese gene. *Science (New York, N.Y.)*, 269(5223), 543–546. <https://doi.org/10.1126/science.7624777>
- Halvorson, I., Gregor, L., & Thornhill, J. A. (1990). Brown adipose tissue thermogenesis is activated by electrical and chemical (L-glutamate) stimulation of the ventromedial hypothalamic nucleus in cold-acclimated rats. *Brain research*, 522(1), 76–82. [https://doi.org/10.1016/0006-8993\(90\)91579-6](https://doi.org/10.1016/0006-8993(90)91579-6)
- Hanani, M., & Spray, D. C. (2020). Emerging importance of satellite glia in nervous system function and dysfunction. *Nature reviews. Neuroscience*, 21(9), 485–498. <https://doi.org/10.1038/s41583-020-0333-z>
- Han, S. M., Baig, H. S., & Hammarlund, M. (2016). Mitochondria Localize to Injured Axons to Support Regeneration. *Neuron*, 92(6), 1308–1323. <https://doi.org/10.1016/j.neuron.2016.11.025>

- Han, Y., He, Y., Harris, L., Xu, Y., & Wu, Q. (2023). Identification of a GABAergic neural circuit governing leptin signaling deficiency-induced obesity. *eLife*, *12*, e82649. <https://doi.org/10.7554/eLife.82649>
- Harlan, S. M., Morgan, D. A., Agassandian, K., Guo, D. F., Cassell, M. D., Sigmund, C. D., Mark, A. L., & Rahmouni, K. (2011). Ablation of the leptin receptor in the hypothalamic arcuate nucleus abrogates leptin-induced sympathetic activation. *Circulation research*, *108*(7), 808–812. <https://doi.org/10.1161/CIRCRESAHA.111.240226>
- Hausman, G. J., Poulos, S. P., Richardson, R. L., Barb, C. R., Andacht, T., Kirk, H. C., & Mynatt, R. L. (2006). Secreted proteins and genes in fetal and neonatal pig adipose tissue and stromal-vascular cells. *Journal of animal science*, *84*(7), 1666–1681. <https://doi.org/10.2527/jas.2005-539>
- Haynes, W. G., Morgan, D. A., Djalali, A., Sivitz, W. I., & Mark, A. L. (1999). Interactions between the melanocortin system and leptin in control of sympathetic nerve traffic. *Hypertension (Dallas, Tex. : 1979)*, *33*(1 Pt 2), 542–547. <https://doi.org/10.1161/01.hyp.33.1.542>
- Hill, J. O., Wyatt, H. R., & Peters, J. C. (2012). Energy balance and obesity. *Circulation*, *126*(1), 126–132. <https://doi.org/10.1161/CIRCULATIONAHA.111.087213>
- Himms-Hagen, J., Cui, J., Danforth, E., Jr, Taatjes, D. J., Lang, S. S., Waters, B. L., & Claus, T. H. (1994). Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. *The American journal of physiology*, *266*(4 Pt 2), R1371–R1382. <https://doi.org/10.1152/ajpregu.1994.266.4.R1371>
- Hücking, K., Hamilton-Wessler, M., Ellmerer, M., & Bergman, R. N. (2003). Burst-like control of lipolysis by the sympathetic nervous system in vivo. *The Journal of clinical investigation*, *111*(2), 257–264. <https://doi.org/10.1172/JCI14466>
- Huesing, C., Qualls-Creekmore, E., Lee, N., François, M., Torres, H., Zhang, R., Burk, D. H., Yu, S., Morrison, C. D., Berthoud, H. R., Neuhuber, W., & Münzberg, H. (2021). Sympathetic innervation of inguinal white adipose tissue in the mouse. *The Journal of comparative neurology*, *529*(7), 1465–1485. <https://doi.org/10.1002/cne.25031>

- Ikeda, K., Kang, Q., Yoneshiro, T., Camporez, J. P., Maki, H., Homma, M., Shinoda, K., Chen, Y., Lu, X., Maretich, P., Tajima, K., Ajuwon, K. M., Soga, T., & Kajimura, S. (2017). UCP1-independent signaling involving SERCA2b-mediated calcium cycling regulates beige fat thermogenesis and systemic glucose homeostasis. *Nature medicine*, 23(12), 1454–1465. <https://doi.org/10.1038/nm.4429>
- Ingalls, A. M., Dickie, M. M. & Snell, G. D. (1950) Obese, a new mutation in the house mouse. *The Journal of heredity*, 41(12), 317–318. <https://doi.org/10.1093/oxfordjournals.jhered.a106073>
- Inokuma, K., Ogura-Okamatsu, Y., Toda, C., Kimura, K., Yamashita, H., & Saito, M. (2005). Uncoupling protein 1 is necessary for norepinephrine-induced glucose utilization in brown adipose tissue. *Diabetes*, 54(5), 1385–1391. <https://doi.org/10.2337/diabetes.54.5.1385>
- Jiang, H., Ding, X., Cao, Y., Wang, H., & Zeng, W. (2017). Dense Intra-adipose Sympathetic Arborizations Are Essential for Cold-Induced Beiging of Mouse White Adipose Tissue. *Cell metabolism*, 26(4), 686–692.e3. <https://doi.org/10.1016/j.cmet.2017.08.016>
- Jimenez, M., Barbatelli, G., Allevi, R., Cinti, S., Seydoux, J., Giacobino, J. P., Muzzin, P., & Preitner, F. (2003). Beta 3-adrenoceptor knockout in C57BL/6J mice depresses the occurrence of brown adipocytes in white fat. *European journal of biochemistry*, 270(4), 699–705. <https://doi.org/10.1046/j.1432-1033.2003.03422.x>
- Jones, D. D., Ramsay, T. G., Hausman, G. J., & Martin, R. J. (1992). Norepinephrine inhibits rat pre-adipocyte proliferation. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity*, 16(5), 349–354.
- Kajimura, S., Seale, P., & Spiegelman, B. M. (2010). Transcriptional control of brown fat development. *Cell metabolism*, 11(4), 257–262. <https://doi.org/10.1016/j.cmet.2010.03.005>
- Kazak, L., Chouchani, E. T., Jedrychowski, M. P., Erickson, B. K., Shinoda, K., Cohen, P., Vetrivelan, R., Lu, G. Z., Laznik-Bogoslavski, D., Hasenfuss, S. C., Kajimura, S., Gygi, S. P., & Spiegelman,

- B. M. (2015). A creatine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat. *Cell*, 163(3), 643–655. <https://doi.org/10.1016/j.cell.2015.09.035>
- Kim, K. W., Sohn, J. W., Kohno, D., Xu, Y., Williams, K., & Elmquist, J. K. (2011). SF-1 in the ventral medial hypothalamic nucleus: a key regulator of homeostasis. *Molecular and cellular endocrinology*, 336(1-2), 219–223. <https://doi.org/10.1016/j.mce.2010.11.019>
- Kim, S. N., Jung, Y. S., Kwon, H. J., Seong, J. K., Granneman, J. G., & Lee, Y. H. (2016). Sex differences in sympathetic innervation and browning of white adipose tissue of mice. *Biology of sex differences*, 7, 67. <https://doi.org/10.1186/s13293-016-0121-7>
- Koivisto, V. A., Nikkilä, E. A., & Akerblom, H. K. (1975). Influence of norepinephrine and exercise on lipolysis in adipose tissue of diabetic rats. *Diabetologia*, 11(5), 401–405. <https://doi.org/10.1007/BF00429907>
- Komori, T., Morikawa, Y., Nanjo, K., & Senba, E. (2006). Induction of brain-derived neurotrophic factor by leptin in the ventromedial hypothalamus. *Neuroscience*, 139(3), 1107–1115. <https://doi.org/10.1016/j.neuroscience.2005.12.066>
- Kong, D., Tong, Q., Ye, C., Koda, S., Fuller, P. M., Krashes, M. J., Vong, L., Ray, R. S., Olson, D. P., & Lowell, B. B. (2012). GABAergic RIP-Cre neurons in the arcuate nucleus selectively regulate energy expenditure. *Cell*, 151(3), 645–657. <https://doi.org/10.1016/j.cell.2012.09.020>
- Kotz, C. M., Wang, C. F., Briggs, J. E., Levine, A. S., & Billington, C. J. (2000). Effect of NPY in the hypothalamic paraventricular nucleus on uncoupling proteins 1, 2, and 3 in the rat. *American journal of physiology. Regulatory, integrative and comparative physiology*, 278(2), R494–R498. <https://doi.org/10.1152/ajpregu.2000.278.2.R494>
- Labbé, S. M., Caron, A., Festuccia, W. T., Lecomte, R., & Richard, D. (2018). Interscapular brown adipose tissue denervation does not promote the oxidative activity of inguinal white adipose tissue in male mice. *American journal of physiology. Endocrinology and metabolism*, 315(5), E815–E824. <https://doi.org/10.1152/ajpendo.00210.2018>

- Landry, G. J., Kent, B. A., Patton, D. F., Jaholkowski, M., Marchant, E. G., & Mistlberger, R. E. (2011). Evidence for time-of-day dependent effect of neurotoxic dorsomedial hypothalamic lesions on food anticipatory circadian rhythms in rats. *PloS one*, *6*(9), e24187. <https://doi.org/10.1371/journal.pone.0024187>
- Langlet, F., Mullier, A., Bouret, S. G., Prevot, V., & Dehouck, B. (2013). Tanycyte-like cells form a blood-cerebrospinal fluid barrier in the circumventricular organs of the mouse brain. *The Journal of comparative neurology*, *521*(15), 3389–3405. <https://doi.org/10.1002/cne.23355>
- Levin, N., Nelson, C., Gurney, A., Vandlen, R., & de Sauvage, F. (1996). Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(4), 1726–1730. <https://doi.org/10.1073/pnas.93.4.1726>
- Löllmann, B., Grüninger, S., Stricker-Krongrad, A., & Chiesi, M. (1997). Detection and quantification of the leptin receptor splice variants Ob-Ra, b, and, e in different mouse tissues. *Biochemical and biophysical research communications*, *238*(2), 648–652. <https://doi.org/10.1006/bbrc.1997.7205>
- Lopez-Valpuesta, F. J., Nyce, J. W., & Myers, R. D. (1996). NPY-Y1 receptor antisense injected centrally in rats causes hyperthermia and feeding. *Neuroreport*, *7*(15-17), 2781–2784. <https://doi.org/10.1097/00001756-199611040-00075>
- Lundberg, J. M., Franco-Cereceda, A., Hemsén, A., Lacroix, J. S., & Pernow, J. (1990). Pharmacology of noradrenaline and neuropeptide tyrosine (NPY)-mediated sympathetic cotransmission. *Fundamental & clinical pharmacology*, *4*(4), 373–391. <https://doi.org/10.1111/j.1472-8206.1990.tb00692.x>
- Luo, X., Ribeiro, M., Bray, E. R., Lee, D. H., Yungher, B. J., Mehta, S. T., Thakor, K. A., Diaz, F., Lee, J. K., Moraes, C. T., Bixby, J. L., Lemmon, V. P., & Park, K. K. (2016). Enhanced Transcriptional Activity and Mitochondrial Localization of STAT3 Co-induce Axon Regrowth in the Adult Central Nervous System. *Cell reports*, *15*(2), 398–410. <https://doi.org/10.1016/j.celrep.2016.03.029>

- Lyons, C. E., Razzoli, M., Larson, E., Svedberg, D., Frontini, A., Cinti, S., Vulchanova, L., Sanders, M., Thomas, M., & Bartolomucci, A. (2020). Optogenetic-induced sympathetic neuromodulation of brown adipose tissue thermogenesis. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, *34*(2), 2765–2773.
<https://doi.org/10.1096/fj.201901361RR>
- Madisen, L., Zwingman, T. A., Sunkin, S. M., Oh, S. W., Zariwala, H. A., Gu, H., Ng, L. L., Palmiter, R. D., Hawrylycz, M. J., Jones, A. R., Lein, E. S., & Zeng, H. (2010). A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nature neuroscience*, *13*(1), 133–140. <https://doi.org/10.1038/nn.2467>
- Maickel, R. P., Stern, D. N., Takabatake, E., & Brodie, B. B. (1967). The sympathetic nervous system as a homeostatic mechanism. II. Effect of adrenocortical hormones on body temperature maintenance of cold-exposed adrenalectomized rats. *The Journal of pharmacology and experimental therapeutics*, *157*(1), 111–116.
- Makita, T., Sucov, H. M., Garipey, C. E., Yanagisawa, M., & Ginty, D. D. (2008). Endothelins are vascular-derived axonal guidance cues for developing sympathetic neurons. *Nature*, *452*(7188), 759–763. <https://doi.org/10.1038/nature06859>
- Martinez-Sanchez, N., Sweeney, O., Sidarta-Oliveira, D., Caron, A., Stanley, S. A., & Domingos, A. I. (2022). The sympathetic nervous system in the 21st century: Neuroimmune interactions in metabolic homeostasis and obesity. *Neuron*, *110*(21), 3597–3626.
<https://doi.org/10.1016/j.neuron.2022.10.017>
- Mattsson, C. L., Csikasz, R. I., Chernogubova, E., Yamamoto, D. L., Hogberg, H. T., Amri, E. Z., Hutchinson, D. S., & Bengtsson, T. (2011). β_1 -Adrenergic receptors increase UCP1 in human MADS brown adipocytes and rescue cold-acclimated β_3 -adrenergic receptor-knockout mice via nonshivering thermogenesis. *American journal of physiology. Endocrinology and metabolism*, *301*(6), E1108–E1118. <https://doi.org/10.1152/ajpendo.00085.2011>

- Masuo, K., Straznicky, N. E., Lambert, G. W., Katsuya, T., Sugimoto, K., Rakugi, H., ... Esler, M. D. (2008). Leptin-Receptor Polymorphisms Relate to Obesity through Blunted Leptin-Mediated Sympathetic Nerve Activation in a Caucasian Male Population. *Hypertension Research*, 31(6), 1093–1100. <https://doi.org/10.1291/hypres.31.1093>
- Mehta, S. T., Luo, X., Park, K. K., Bixby, J. L., & Lemmon, V. P. (2016). Hyperactivated Stat3 boosts axon regeneration in the CNS. *Experimental neurology*, 280, 115–120. <https://doi.org/10.1016/j.expneurol.2016.03.004>
- Miller, S. M., Schmalz, P. F., Benarroch, E. E., & Szurszewski, J. H. (1999). Leptin receptor immunoreactivity in sympathetic prevertebral ganglion neurons of mouse and rat. *Neuroscience letters*, 265(2), 75–78. [https://doi.org/10.1016/s0304-3940\(99\)00215-3](https://doi.org/10.1016/s0304-3940(99)00215-3)
- Minokoshi, Y., Haque, M. S., & Shimazu, T. (1999). Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. *Diabetes*, 48(2), 287–291. <https://doi.org/10.2337/diabetes.48.2.287>
- Monda, M., Sullo, A., & De Luca, B. (1997). Lesions of the ventromedial hypothalamus reduce postingestional thermogenesis. *Physiology & behavior*, 61(5), 687–691. [https://doi.org/10.1016/s0031-9384\(96\)00520-3](https://doi.org/10.1016/s0031-9384(96)00520-3)
- Mottillo, E. P., Balasubramanian, P., Lee, Y. H., Weng, C., Kershaw, E. E., & Granneman, J. G. (2014). Coupling of lipolysis and de novo lipogenesis in brown, beige, and white adipose tissues during chronic β 3-adrenergic receptor activation. *Journal of lipid research*, 55(11), 2276–2286. <https://doi.org/10.1194/jlr.M050005>
- Mountoufaris, G., Chen, W. V., Hirabayashi, Y., O'Keeffe, S., Chevee, M., Nwakeze, C. L., Polleux, F., & Maniatis, T. (2017). Multicluster Pcdh diversity is required for mouse olfactory neural circuit assembly. *Science (New York, N.Y.)*, 356(6336), 411–414. <https://doi.org/10.1126/science.aai8801>

Münzberg, H., Qualls-Creekmore, E., Berthoud, H. R., Morrison, C. D., & Yu, S. (2016). Neural Control of Energy Expenditure. *Handbook of experimental pharmacology*, 233, 173–194.

https://doi.org/10.1007/164_2015_33

Nakagawa, T., Tsuchida, A., Itakura, Y., Nonomura, T., Ono, M., Hirota, F., Inoue, T., Nakayama, C., Taiji, M., & Noguchi, H. (2000). Brain-derived neurotrophic factor regulates glucose metabolism by modulating energy balance in diabetic mice. *Diabetes*, 49(3), 436–444.

<https://doi.org/10.2337/diabetes.49.3.436>

Nakagomi, A., Okada, S., Yokoyama, M., Yoshida, Y., Shimizu, I., Miki, T., Kobayashi, Y., & Minamino, T. (2015). Role of the central nervous system and adipose tissue BDNF/TrkB axes in metabolic regulation. *NPJ aging and mechanisms of disease*, 1, 15009.

<https://doi.org/10.1038/npjamd.2015.9>

Nakamura, Y., Yanagawa, Y., Morrison, S. F., & Nakamura, K. (2017). Medullary Reticular Neurons Mediate Neuropeptide Y-Induced Metabolic Inhibition and Mastication. *Cell metabolism*, 25(2), 322–334. <https://doi.org/10.1016/j.cmet.2016.12.002>

Nguyen, N. L., Barr, C. L., Ryu, V., Cao, Q., Xue, B., & Bartness, T. J. (2017). Separate and shared sympathetic outflow to white and brown fat coordinately regulates thermoregulation and beige adipocyte recruitment. *American journal of physiology. Regulatory, integrative and comparative physiology*, 312(1), R132–R145. <https://doi.org/10.1152/ajpregu.00344.2016>

Nonomura, T., Tsuchida, A., Ono-Kishino, M., Nakagawa, T., Taiji, M., & Noguchi, H. (2001). Brain-derived neurotrophic factor regulates energy expenditure through the central nervous system in obese diabetic mice. *International journal of experimental diabetes research*, 2(3), 201–209.

<https://doi.org/10.1155/edr.2001.201>

Nour, T. Y., & Altıntaş, K. H. (2023). Effect of the COVID-19 pandemic on obesity and its risk factors: a systematic review. *BMC public health*, 23(1), 1018. [https://doi.org/10.1186/s12889-023-15833-](https://doi.org/10.1186/s12889-023-15833-2)

- Obradovic, M., Sudar-Milovanovic, E., Soskic, S., Essack, M., Arya, S., Stewart, A. J., Gojobori, T., & Isenovic, E. R. (2021). Leptin and Obesity: Role and Clinical Implication. *Frontiers in endocrinology*, *12*, 585887. <https://doi.org/10.3389/fendo.2021.585887>
- Orava, J., Nuutila, P., Lidell, M. E., Oikonen, V., Noponen, T., Viljanen, T., Scheinin, M., Taittonen, M., Niemi, T., Enerbäck, S., & Virtanen, K. A. (2011). Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell metabolism*, *14*(2), 272–279. <https://doi.org/10.1016/j.cmet.2011.06.012>
- Pancho, A., Aerts, T., Mitsogiannis, M. D., & Seuntjens, E. (2020). Protocadherins at the Crossroad of Signaling Pathways. *Frontiers in molecular neuroscience*, *13*, 117. <https://doi.org/10.3389/fnmol.2020.00117>
- Paul, M. J., Freeman, D. A., Park, J. H., & Dark, J. (2005). Neuropeptide Y induces torpor-like hypothermia in Siberian hamsters. *Brain research*, *1055*(1-2), 83–92. <https://doi.org/10.1016/j.brainres.2005.06.090>
- Peeraully, M. R., Jenkins, J. R., & Trayhurn, P. (2004). NGF gene expression and secretion in white adipose tissue: regulation in 3T3-L1 adipocytes by hormones and inflammatory cytokines. *American journal of physiology. Endocrinology and metabolism*, *287*(2), E331–E339. <https://doi.org/10.1152/ajpendo.00076.2004>
- Pellegrino, M. J., McCully, B. H., & Habecker, B. A. (2014). Leptin stimulates sympathetic axon outgrowth. *Neuroscience letters*, *566*, 1–5. <https://doi.org/10.1016/j.neulet.2014.02.014>
- Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T., & Collins, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science (New York, N.Y.)*, *269*(5223), 540–543. <https://doi.org/10.1126/science.7624776>
- Perry, R. J., Lyu, K., Rabin-Court, A., Dong, J., Li, X., Yang, Y., Qing, H., Wang, A., Yang, X., & Shulman, G. I. (2020). Leptin mediates postprandial increases in body temperature through hypothalamus-adrenal medulla-adipose tissue crosstalk. *The Journal of clinical investigation*, *130*(4), 2001–2016. <https://doi.org/10.1172/JCI134699>

- Pernet, V., Joly, S., Jordi, N., Dalkara, D., Guzik-Kornacka, A., Flannery, J. G., & Schwab, M. E. (2013). Misguidance and modulation of axonal regeneration by Stat3 and Rho/ROCK signaling in the transparent optic nerve. *Cell death & disease*, *4*(7), e734. <https://doi.org/10.1038/cddis.2013.266>
- Pinto, S., Roseberry, A. G., Liu, H., Diano, S., Shanabrough, M., Cai, X., Friedman, J. M., & Horvath, T. L. (2004). Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science (New York, N.Y.)*, *304*(5667), 110–115. <https://doi.org/10.1126/science.1089459>
- Preite, N. Z., Nascimento, B. P., Muller, C. R., Américo, A. L., Higa, T. S., Evangelista, F. S., Lancellotti, C. L., Henriques, F. S., Batista, M. L., Jr, Bianco, A. C., & Ribeiro, M. O. (2016). Disruption of beta3 adrenergic receptor increases susceptibility to DIO in mouse. *The Journal of endocrinology*, *231*(3), 259–269. <https://doi.org/10.1530/JOE-16-0199>
- Puente-Ruiz, S. C., & Jais, A. (2022). Reciprocal signaling between adipose tissue depots and the central nervous system. *Frontiers in cell and developmental biology*, *10*, 979251. <https://doi.org/10.3389/fcell.2022.979251>
- Qiu, J., Fang, Y., Rønnekleiv, O. K., & Kelly, M. J. (2010). Leptin excites proopiomelanocortin neurons via activation of TRPC channels. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *30*(4), 1560–1565. <https://doi.org/10.1523/JNEUROSCI.4816-09.2010>
- Raefsky, S. M., & Mattson, M. P. (2017). Adaptive responses of neuronal mitochondria to bioenergetic challenges: Roles in neuroplasticity and disease resistance. *Free radical biology & medicine*, *102*, 203–216. <https://doi.org/10.1016/j.freeradbiomed.2016.11.045>
- Rau, A. R., & Hentges, S. T. (2017). The Relevance of AgRP Neuron-Derived GABA Inputs to POMC Neurons Differs for Spontaneous and Evoked Release. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *37*(31), 7362–7372. <https://doi.org/10.1523/JNEUROSCI.0647-17.2017>
- Rezai-Zadeh, K., Yu, S., Jiang, Y., Laque, A., Schwartzburg, C., Morrison, C. D., Derbenev, A. V., Zsombok, A., & Münzberg, H. (2014). Leptin receptor neurons in the dorsomedial hypothalamus

- are key regulators of energy expenditure and body weight, but not food intake. *Molecular metabolism*, 3(7), 681–693. <https://doi.org/10.1016/j.molmet.2014.07.008>
- Richard AJ, White U, Elks CM, et al. Adipose Tissue: Physiology to Metabolic Dysfunction. [Updated 2020 Apr 4]. In: Feingold KR, Anawalt B, Blackman MR, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK555602/>
- Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., Lechan, R. M., & Jaenisch, R. (2001). Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Molecular endocrinology (Baltimore, Md.)*, 15(10), 1748–1757. <https://doi.org/10.1210/mend.15.10.0706>
- Rosell, M., Kaforou, M., Frontini, A., Okolo, A., Chan, Y. W., Nikolopoulou, E., Millership, S., Fenech, M. E., MacIntyre, D., Turner, J. O., Moore, J. D., Blackburn, E., Gullick, W. J., Cinti, S., Montana, G., Parker, M. G., & Christian, M. (2014). Brown and white adipose tissues: intrinsic differences in gene expression and response to cold exposure in mice. *American journal of physiology. Endocrinology and metabolism*, 306(8), E945–E964. <https://doi.org/10.1152/ajpendo.00473.2013>
- Rosen, E. D., & Spiegelman, B. M. (2000). Molecular regulation of adipogenesis. *Annual review of cell and developmental biology*, 16, 145–171. <https://doi.org/10.1146/annurev.cellbio.16.1.145>
- Ryu, V., & Buettner, C. (2019). Fat cells gobbling up norepinephrine?. *PLoS biology*, 17(2), e3000138. <https://doi.org/10.1371/journal.pbio.3000138>
- Satoh, N., Ogawa, Y., Katsuura, G., Hayase, M., Tsuji, T., Imagawa, K., Yoshimasa, Y., Nishi, S., Hosoda, K., & Nakao, K. (1997). The arcuate nucleus as a primary site of satiety effect of leptin in rats. *Neuroscience letters*, 224(3), 149–152. [https://doi.org/10.1016/S0304-3940\(97\)00163-8](https://doi.org/10.1016/S0304-3940(97)00163-8)
- Satoh, N., Ogawa, Y., Katsuura, G., Numata, Y., Tsuji, T., Hayase, M., Ebihara, K., Masuzaki, H., Hosoda, K., Yoshimasa, Y., & Nakao, K. (1999). Sympathetic activation of leptin via the

- ventromedial hypothalamus: leptin-induced increase in catecholamine secretion. *Diabetes*, 48(9), 1787–1793. <https://doi.org/10.2337/diabetes.48.9.1787>
- Savitt, J. M., Jang, S. S., Mu, W., Dawson, V. L., & Dawson, T. M. (2005). Bcl-x is required for proper development of the mouse substantia nigra. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25(29), 6721–6728. <https://doi.org/10.1523/JNEUROSCI.0760-05.2005>
- Schena, G., & Caplan, M. J. (2019). Everything You Always Wanted to Know about β 3-AR * (* But Were Afraid to Ask). *Cells*, 8(4), 357. <https://doi.org/10.3390/cells8040357>
- Schreiber, R., Diwoky, C., Schoiswohl, G., Feiler, U., Wongsiriroj, N., Abdellatif, M., Kolb, D., Hoeks, J., Kershaw, E. E., Sedej, S., Schrauwen, P., Haemmerle, G., & Zechner, R. (2017). Cold-Induced Thermogenesis Depends on ATGL-Mediated Lipolysis in Cardiac Muscle, but Not Brown Adipose Tissue. *Cell metabolism*, 26(5), 753–763.e7. <https://doi.org/10.1016/j.cmet.2017.09.004>
- Scott-Solomon, E., Boehm, E. & Kuruvilla, R. (2021). The sympathetic nervous system in development and disease. *Nat Rev Neurosci* 22, 685–702. <https://doi.org/10.1038/s41583-021-00523-y>
- Selvaraj, B. T., Frank, N., Bender, F. L., Asan, E., & Sendtner, M. (2012). Local axonal function of STAT3 rescues axon degeneration in the pmn model of motoneuron disease. *The Journal of cell biology*, 199(3), 437–451. <https://doi.org/10.1083/jcb.201203109>
- Serradeil-Le Gal, C., Lafontan, M., Raufaste, D., Marchand, J., Pouzet, B., Casellas, P., Pascal, M., Maffrand, J. P., & Le Fur, G. (2000). Characterization of NPY receptors controlling lipolysis and leptin secretion in human adipocytes. *FEBS letters*, 475(2), 150–156. [https://doi.org/10.1016/s0014-5793\(00\)01649-5](https://doi.org/10.1016/s0014-5793(00)01649-5)
- Shanley, L. J., Irving, A. J., Rae, M. G., Ashford, M. L., & Harvey, J. (2002). Leptin inhibits rat hippocampal neurons via activation of large conductance calcium-activated K⁺ channels. *Nature neuroscience*, 5(4), 299–300. <https://doi.org/10.1038/nn824>
- Shin, H., Ma, Y., Chanturiya, T., Cao, Q., Wang, Y., Kadegowda, A. K. G., Jackson, R., Rumore, D., Xue, B., Shi, H., Gavrilova, O., & Yu, L. (2017). Lipolysis in Brown Adipocytes Is Not Essential

- for Cold-Induced Thermogenesis in Mice. *Cell metabolism*, 26(5), 764–777.e5.
<https://doi.org/10.1016/j.cmet.2017.09.002>
- Shi, Y. C., Lau, J., Lin, Z., Zhang, H., Zhai, L., Sperk, G., Heilbronn, R., Mietzsch, M., Weger, S., Huang, X. F., Enriquez, R. F., Baldock, P. A., Zhang, L., Sainsbury, A., Herzog, H., & Lin, S. (2013). Arcuate NPY controls sympathetic output and BAT function via a relay of tyrosine hydroxylase neurons in the PVN. *Cell metabolism*, 17(2), 236–248.
<https://doi.org/10.1016/j.cmet.2013.01.006>
- Simonds, S. E., Pryor, J. T., Ravussin, E., Greenway, F. L., Dileone, R., Allen, A. M., Bassi, J., Elmquist, J. K., Keogh, J. M., Henning, E., Myers, M. G., Jr, Licinio, J., Brown, R. D., Enriori, P. J., O'Rahilly, S., Sternson, S. M., Grove, K. L., Spanswick, D. C., Farooqi, I. S., & Cowley, M. A. (2014). Leptin mediates the increase in blood pressure associated with obesity. *Cell*, 159(6), 1404–1416. <https://doi.org/10.1016/j.cell.2014.10.058>
- Smith, G. M., & Gallo, G. (2018). The role of mitochondria in axon development and regeneration. *Developmental neurobiology*, 78(3), 221–237. <https://doi.org/10.1002/dneu.22546>
- Spanswick, D., Smith, M. A., Groppi, V. E., Logan, S. D., & Ashford, M. L. (1997). Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature*, 390(6659), 521–525. <https://doi.org/10.1038/37379>
- Steppan, C. M., & Swick, A. G. (1999). A role for leptin in brain development. *Biochemical and biophysical research communications*, 256(3), 600–602. <https://doi.org/10.1006/bbrc.1999.0382>
- Stierman, Bryan et al. (2021). National Health and Nutrition Examination Survey 2017–March 2020 Prepandemic Data Files Development of Files and Prevalence Estimates for Selected Health Outcomes. (158). <https://stacks.cdc.gov/view/cdc/106273>
- Susulic, V. S., Frederich, R. C., Lawitts, J., Tozzo, E., Kahn, B. B., Harper, M. E., Himms-Hagen, J., Flier, J. S., & Lowell, B. B. (1995). Targeted disruption of the beta 3-adrenergic receptor gene. *The Journal of biological chemistry*, 270(49), 29483–29492.
<https://doi.org/10.1074/jbc.270.49.29483>

- Su, Y., Zhang, W., Patro, C. P. K., Zhao, J., Mu, T., Ma, Z., Xu, J., Ban, K., Yi, C., & Zhou, Y. (2020). STAT3 Regulates Mouse Neural Progenitor Proliferation and Differentiation by Promoting Mitochondrial Metabolism. *Frontiers in cell and developmental biology*, 8, 362. <https://doi.org/10.3389/fcell.2020.00362>
- Szczepanek, K., Chen, Q., Derecka, M., Salloum, F. N., Zhang, Q., Szelag, M., Cichy, J., Kukreja, R. C., Dulak, J., Lesniewski, E. J., & Larner, A. C. (2011). Mitochondrial-targeted Signal transducer and activator of transcription 3 (STAT3) protects against ischemia-induced changes in the electron transport chain and the generation of reactive oxygen species. *The Journal of biological chemistry*, 286(34), 29610–29620. <https://doi.org/10.1074/jbc.M111.226209>
- Székely, M., Pétervári, E., Pákai, E., Hummel, Z., & Szelényi, Z. (2005). Acute, subacute and chronic effects of central neuropeptide Y on energy balance in rats. *Neuropeptides*, 39(2), 103–115. <https://doi.org/10.1016/j.npep.2005.01.005>
- Takahashi, A., Shimazu, T., & Maruyama, Y. (1992). Importance of sympathetic nerves for the stimulatory effect of cold exposure on glucose utilization in brown adipose tissue. *The Japanese journal of physiology*, 42(4), 653–664. <https://doi.org/10.2170/jjphysiol.42.653>
- Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G. J., Campfield, L. A., Clark, F. T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K. J., Smutko, J. S., Mays, G. G., Wool, E. A., Monroe, C. A., & Tepper, R. I. (1995). Identification and expression cloning of a leptin receptor, OB-R. *Cell*, 83(7), 1263–1271. [https://doi.org/10.1016/0092-8674\(95\)90151-5](https://doi.org/10.1016/0092-8674(95)90151-5)
- Tiwari A, Balasundaram P. Public Health Considerations Regarding Obesity. [Updated 2023 Jun 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK572122/>
- Ueta, C. B., Fernandes, G. W., Capelo, L. P., Fonseca, T. L., Maculan, F. D., Gouveia, C. H., Brum, P. C., Christoffolete, M. A., Aoki, M. S., Lancellotti, C. L., Kim, B., Bianco, A. C., & Ribeiro, M.

- O. (2012). $\beta(1)$ Adrenergic receptor is key to cold- and diet-induced thermogenesis in mice. *The Journal of endocrinology*, 214(3), 359–365. <https://doi.org/10.1530/JOE-12-0155>
- Unger, T. J., Calderon, G. A., Bradley, L. C., Sena-Esteves, M., & Rios, M. (2007). Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(52), 14265–14274. <https://doi.org/10.1523/JNEUROSCI.3308-07.2007>
- Vaisse, C., Halaas, J. L., Horvath, C. M., Darnell, J. E., Jr, Stoffel, M., & Friedman, J. M. (1996). Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nature genetics*, 14(1), 95–97. <https://doi.org/10.1038/ng0996-95>
- Van den Berg, S. M., van Dam, A. D., Rensen, P. C., de Winther, M. P., & Lutgens, E. (2017). Immune Modulation of Brown(ing) Adipose Tissue in Obesity. *Endocrine reviews*, 38(1), 46–68. <https://doi.org/10.1210/er.2016-1066>
- Villanueva, E. C., & Myers, M. G. (2008). Leptin receptor signaling and the regulation of mammalian physiology. *International Journal of Obesity*, 32(S7). <https://doi.org/10.1038/ijo.2008.232>
- Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerbäck, S., & Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *The New England journal of medicine*, 360(15), 1518–1525. <https://doi.org/10.1056/NEJMoa0808949>
- von Essen, G., Lindsund, E., Cannon, B., & Nedergaard, J. (2017). Adaptive facultative diet-induced thermogenesis in wild-type but not in UCP1-ablated mice. *American journal of physiology. Endocrinology and metabolism*, 313(5), E515–E527. <https://doi.org/10.1152/ajpendo.00097.2017>
- Xu, B., Zheng, C., Chen, X., Zhang, Z., Liu, J., Spencer, P., & Yang, X. (2019). Dysregulation of Myosin Complex and Striated Muscle Contraction Pathway in the Brains of ALS-SOD1 Model Mice. *ACS chemical neuroscience*, 10(5), 2408–2417. <https://doi.org/10.1021/acscemneuro.8b00704>

- Xu, J., Bartolome, C. L., Low, C. S., Yi, X., Chien, C. H., Wang, P., & Kong, D. (2018). Genetic identification of leptin neural circuits in energy and glucose homeostases. *Nature*, *556*(7702), 505–509. <https://doi.org/10.1038/s41586-018-0049-7>
- Xu, Y., Kim, E. R., Zhao, R., Myers, M. G., Jr, Munzberg, H., & Tong, Q. (2013). Glutamate release mediates leptin action on energy expenditure. *Molecular metabolism*, *2*(2), 109–115. <https://doi.org/10.1016/j.molmet.2013.01.004>
- Xue, B., Rim, J. S., Hogan, J. C., Coulter, A. A., Koza, R. A., & Kozak, L. P. (2007). Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat. *Journal of lipid research*, *48*(1), 41–51. <https://doi.org/10.1194/jlr.M600287-JLR200>
- Yang, M., Liu, S., & Zhang, C. (2022). The Related Metabolic Diseases and Treatments of Obesity. *Healthcare (Basel, Switzerland)*, *10*(9), 1616. <https://doi.org/10.3390/healthcare10091616>
- Yoo, S., Cha, D., Kim, D. W., Hoang, T. V., & Blackshaw, S. (2019). Tanycyte-Independent Control of Hypothalamic Leptin Signaling. *Frontiers in neuroscience*, *13*, 240. <https://doi.org/10.3389/fnins.2019.00240>
- Yoo, S., Cha, D., Kim, S., Jiang, L., Cooke, P., Adebisin, M., Wolfe, A., Riddle, R., Aja, S., & Blackshaw, S. (2020). Tanycyte ablation in the arcuate nucleus and median eminence increases obesity susceptibility by increasing body fat content in male mice. *Glia*, *68*(10), 1987–2000. <https://doi.org/10.1002/glia.23817>
- Youngstrom, T. G., & Bartness, T. J. (1995). Catecholaminergic innervation of white adipose tissue in Siberian hamsters. *The American journal of physiology*, *268*(3 Pt 2), R744–R751. <https://doi.org/10.1152/ajpregu.1995.268.3.R744>
- Yu, S., François, M., Huesing, C., & Münzberg, H. (2018). The Hypothalamic Preoptic Area and Body Weight Control. *Neuroendocrinology*, *106*(2), 187–194. <https://doi.org/10.1159/000479875>
- Yunes-Medina, L., Paciorowski, A., Nuzbrokh, Y., & Johnson, G. V. W. (2018). Depletion of transglutaminase 2 in neurons alters expression of extracellular matrix and signal transduction

- genes and compromises cell viability. *Molecular and cellular neurosciences*, 86, 72–80.
<https://doi.org/10.1016/j.mcn.2017.11.011>
- Wang, C. S., Kavalali, E. T., & Monteggia, L. M. (2022). BDNF signaling in context: From synaptic regulation to psychiatric disorders. *Cell*, 185(1), 62–76. <https://doi.org/10.1016/j.cell.2021.12.003>
- Wang, P., Loh, K. H., Wu, M., Morgan, D. A., Schneeberger, M., Yu, X., Chi, J., Kosse, C., Kim, D., Rahmouni, K., Cohen, P., & Friedman, J. (2020). A leptin-BDNF pathway regulating sympathetic innervation of adipose tissue. *Nature*, 583(7818), 839–844. <https://doi.org/10.1038/s41586-020-2527-y>
- Wang, W., & Seale, P. (2016). Control of brown and beige fat development. *Nature reviews. Molecular cell biology*, 17(11), 691–702. <https://doi.org/10.1038/nrm.2016.96>
- Wegrzyn, J., Potla, R., Chwae, Y. J., Sepuri, N. B., Zhang, Q., Koeck, T., Derecka, M., Szczepanek, K., Szelag, M., Gornicka, A., Moh, A., Moghaddas, S., Chen, Q., Bobbili, S., Cichy, J., Dulak, J., Baker, D. P., Wolfman, A., Stuehr, D., Hassan, M. O., ... Larner, A. C. (2009). Function of mitochondrial Stat3 in cellular respiration. *Science (New York, N.Y.)*, 323(5915), 793–797.
<https://doi.org/10.1126/science.1164551>
- Wu, J., Boström, P., Sparks, L. M., Ye, L., Choi, J. H., Giang, A. H., Khandekar, M., Virtanen, K. A., Nuutila, P., Schaart, G., Huang, K., Tu, H., van Marken Lichtenbelt, W. D., Hoeks, J., Enerbäck, S., Schrauwen, P., & Spiegelman, B. M. (2012). Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell*, 150(2), 366–376.
<https://doi.org/10.1016/j.cell.2012.05.016>
- Wu, Q., Kazantzis, M., Doege, H., Ortegon, A. M., Tsang, B., Falcon, A., & Stahl, A. (2006). Fatty acid transport protein 1 is required for nonshivering thermogenesis in brown adipose tissue. *Diabetes*, 55(12), 3229–3237. <https://doi.org/10.2337/db06-0749>
- Wu, R., Yu, W., Fu, L., Li, F., Jing, J., Cui, X., Wang, S., Cao, Q., Xue, B., & Shi, H. (2020). Postnatal leptin surge is critical for the transient induction of the developmental beige adipocytes in mice.

- American journal of physiology. Endocrinology and metabolism, 318(4), E453–E461.
<https://doi.org/10.1152/ajpendo.00292.2019>
- Zaia, C. T., Gaziri, L. C., Zaia, D. A., Delattre, E., Dolnikoff, M. S., & Timo-Iaria, C. (1997). Effect of chemical stimulation of the dorsomedial hypothalamic nucleus on blood plasma glucose, triglycerides and free fatty acids in rats. *Brain research bulletin*, 42(3), 195–198.
[https://doi.org/10.1016/s0361-9230\(96\)00225-0](https://doi.org/10.1016/s0361-9230(96)00225-0)
- Zaretskaia, M. V., Zaretsky, D. V., Shekhar, A., & DiMicco, J. A. (2002). Chemical stimulation of the dorsomedial hypothalamus evokes non-shivering thermogenesis in anesthetized rats. *Brain research*, 928(1-2), 113–125. [https://doi.org/10.1016/s0006-8993\(01\)03369-8](https://doi.org/10.1016/s0006-8993(01)03369-8)
- Zeng, W., Pirzgalska, R. M., Pereira, M. M., Kubasova, N., Barateiro, A., Seixas, E., Lu, Y. H., Kozlova, A., Voss, H., Martins, G. G., Friedman, J. M., & Domingos, A. I. (2015). Sympathetic neuro-adipose connections mediate leptin-driven lipolysis. *Cell*, 163(1), 84–94.
<https://doi.org/10.1016/j.cell.2015.08.055>
- Zeng, X., Ye, M., Resch, J. M., Jedrychowski, M. P., Hu, B., Lowell, B. B., Ginty, D. D., & Spiegelman, B. M. (2019). Innervation of thermogenic adipose tissue via a calyntenin 3 β -S100b axis. *Nature*, 569(7755), 229–235. <https://doi.org/10.1038/s41586-019-1156-9>
- Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372(6505), 425–432.
<https://doi.org/10.1038/372425a0>
- Zhou, B., Yu, P., Lin, M. Y., Sun, T., Chen, Y., & Sheng, Z. H. (2016). Facilitation of axon regeneration by enhancing mitochondrial transport and rescuing energy deficits. *The Journal of cell biology*, 214(1), 103–119. <https://doi.org/10.1083/jcb.201605101>
- Zhou, L., & Too, H. P. (2011). Mitochondrial localized STAT3 is involved in NGF induced neurite outgrowth. *PloS one*, 6(6), e21680. <https://doi.org/10.1371/journal.pone.0021680>
- Zhou, X., Gomez-Smith, M., Qin, Z., Duquette, P. M., Cardenas-Blanco, A., Rai, P. S., Harper, M. E., Tsai, E. C., Anisman, H., & Chen, H. H. (2012). Ablation of LMO4 in glutamatergic neurons

impairs leptin control of fat metabolism. *Cellular and molecular life sciences : CMLS*, 69(5), 819–828. <https://doi.org/10.1007/s00018-011-0794-3>