# INVESTIGATING THE ROLE OF INTERLEUKIN-15 IN MODULATING ADIPOSE TISSUE

By

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**ABSTRACT**

Obesity is a major global health concern and is associated with the development of numerous non-communicable diseases. A thorough understanding of the onset of obesity is critical to the development of effective therapeutic strategies against this disease state. Recently, obesity has been described as a complex disease characterized by chronic low grade inflammation. Abnormal adipose tissue expansion is accompanied by an increased presence of proinflammatory immune cells, dysregulated adipokine expression, oxidative stress, and is associated with significant changes in the bacterial composition of the gut. While interleukin-15 (IL-15) has been studied extensively for its immunological effects, this cytokine has recently been shown to influence body weight and fat mass. The focus of this thesis was to elucidate the role of and mechanism by which IL-15 modulates adipose tissue.

Our first study demonstrated that low levels of IL-15 expression are associated with adiposity and promotes an obese state in IL-15-/- mice and human subjects, while IL-15 overexpression was associated with a lean phenotype in IL-15tg mice when compared to appropriate controls. To uncover the underlining mechanisms by which IL-15 mediates differences in body weight, we subsequently determined that IL-15 mediated weight loss occurred independently of lymphocytes. In another study, we showed that IL-15tg mice had increased mitochondrial activity and mass specific to adipose tissue compared to IL-15-/- and B6 mice, while acute IL-15 administration induced the expression of FAO markers in adipose tissue. Lastly, IL-15 treatment

increased mitochondrial membrane potential and decreased lipid deposition in cultured adipocytes, suggesting that IL-15 may mediate its effects directly on adipose tissue. The experimental results presented in this thesis demonstrate that IL-15 is an important regulator of adipose tissue and body weight. Future studies examining the effects of IL-15 on adipose tissue will further our knowledge on IL-15 biology, and may contribute to novel therapeutic strategies for the treatment and prevention of obesity.

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Throughout this process, I have learned a lot about myself. To the readers of this thesis, whether you are starting off your graduate journey or feverishly completing your doctoral dissertation, I leave you with this: Eleanor Roosevelt once said "The future belongs to those who believe in the beauty of their dreams." So I challenge you to work hard, dream big, and know that everything will fall into place. The people acknowledged here did that for me, and I am truly forever grateful.

## PREFACE

This thesis is prepared in the "sandwich" format as outlined in the "Guide for the preparation of Master's and Doctoral Theses" available through the School of Graduate Studies at McMaster University. Chapter 1 of this thesis serves as a general introduction. The body of this thesis consists of 3 chapters (Chapter 2-4), each one an independent study, two of which is published and the other one submitted for publication at the time of the thesis submission. All submitted and published studies and manuscripts included in this thesis were written by the author of this thesis, who is also the first author on all included works. The preamble section preceding each chapter describes the contributions of other authors to the multi-authored work. Finally, the discussion section (Chapter 5) summarizes the conclusions of this thesis and draws out the overall implications.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ADP | Adenosine Diphosphate |
| AMPK | Adenosine Monophosphate (AMP)-activated protein kinase |
| ATM | Adipose tissue macrophages |
| ATP | Adenosine Triphosphate |
| AUC | Area Under the Curve |
| BMI | Body Mass Index |
| CCL | CC-chemokine ligand |
| CCR | CC-chemokine receptor |
| CD | Cluster of Differentiation |
| CHAD | beta-hydroxyacyl CoA dehydrogenase |
| CoA | Coenzyme A |
| CPT-1 | Carnitine Palmitoyltransferase I |
| Cys | Cysteine |
| DCs | Dendritic Cells |
| DEXA | Dual-energy X-ray absorptiometry |
| DMEM | Dulbecco's Modified Eagle Medium |
| DNA | Deoxyribonucleic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| ER | Endoplasmic Reticulum |
| ETC | Electron Transport Chain |
| $FADH_2$ | Flavin Adenine Dinucleotide |
| FAO | Fatty Acid Oxidation |
| FFA | Free Fatty Acid |
| Fiaf | Fasting-Induced Adipose Factor |
| $\gamma_c$ | Gamma Chain |
| GPCR | G-protein-coupled receptor |
| GPx | Glutathione Peroxidase |
| $H_2O_2$ | Hydrogen Peroxide |
| IBMX | 3-isobutyl-1-methylxanthine |
| IFN-γ | Interferon-gamma |
| IL | Interleukin |
| IL-15Rα | Interleukin-15 Receptor-Alpha |
| iNOS | Inducible Nitric Oxide Synthase |
| IRS 1 | Insulin receptor substrate 1 |
| JAK | Janus Kinase |
| JNK | c-Jun NH2-Terminal Kinase |
| LPL | Lipoprotein Lipase |
| LPO | Lipid Peroxidation |
| LPS | Lipopolysaccharide |

| | |
|---|---|
| LSP | Long Signal Peptide |
| MCP-1 | Monocyte Chemoattrant Protein-1 |
| MHC | Major Histocompatibility Complex |
| mRNA | Messenger Ribonucleic acid |
| mtDNA | Mitochondrial Deoxyribonucleic acid |
| NADH | Nicotinamide Adenine Dinucleotide |
| NK | Natural Killer |
| ROS | Reactive Oxygen Species |
| OXPHOS | Oxidative Phosphorylation |
| PAI-1 | Plasminogen Activator Inhibitor–1 |
| PGC-1α/β | Proliferator Activated Receptor (PPAR)-γ Coactivators-1 Alpha/Beta |
| SAPK | Stress-Activated Protein Kinase |
| SGLT-1 | Na+/glucose cotransporter |
| SOD | Superoxide Dismutase |
| SCFAs | Short Chain Fatty Acids |
| SSP | Short Signal Peptide |
| STAT | Signal-Transducer and Activator of Transcription |
| TBARS | Thiobarbituric Acid Reactive Substance |
| TLR | Toll-Like Receptor |
| TNF-α | Tumor Necrosis Factor-Alpha |
| Tregs | Regulatory T cells |
| VLDL | Very Low Density Lipoproteins |
| WHO | World Health Organization |

# CHAPTER 1

# GENERAL INTRODUCTION AND OBJECTIVES

## 1.1 OBESITY

### 1.1.1 The 'Heavy' Burden of Obesity Worldwide

The global community is gaining weight at a rapid pace, resulting in unprecedented prevalence rates of obesity worldwide. Current statistics state more than 1.4 billion adults aged 20 years and older are classified as overweight or obese - a value that has nearly doubled since 1980. Rising overweight and obesity rates afflict low, middle, and high income countries, which are linked to increased mortality compared to underweight-associated deaths worldwide. This amounts to 2.8 million deaths annually and has become the fifth leading risk of global deaths (WHO, 2013). Obesity is a major risk factor for numerous chronic diseases such as musculoskeletal disorders (ie. osteoarthritis), cardiovascular diseases (ie. stroke, high blood pressure, and heart disease), diabetes, mental illness, asthma, and certain forms of cancer such as endometrial, breast and colon (Mokdad et al., 2003; Simon et al., 2006; WHO, 2013). Among rising rates in adults, more than 40 million children under the age of five were overweight, adding significant concern to the health of succeeding generations since reduced life expectancy is predicted to occur for the first time in recent history (Olshansky et al., 2005; WHO, 2013).

Similar to the global burden of disease, current Canadian estimates have reached historic highs with 1 in every 4 Canadians classified as obese, and one third of children aged 5-17 classified as overweight or obese (Gotay et al., 2012; Roberts, Shields, de

Groh, Aziz, & Gilbert, 2012). The Canadian economic cost of obesity is $4.3 billion – a staggering expense that will cripple our health care system as prevalence rates continue to rise (Katzmarzyk & Janssen, 2004). Recently obesity has been defined as a chronic disease requiring medical treatment and prevention (American Medical Association and Council on Science and Public Health, 2013). With this label, governments can now develop a framework to provide accessible treatments targeting obesity, instead of merely treating its complications, with the hope of curbing this epidemic.

### 1.1.2 Defining Obesity

Obesity is a disease simply defined as a state of abnormal or excessive adiposity that may impair health. Although several methods can determine whether an individual is obese, the most common approach is calculating an individual's body mass index (BMI) by dividing their weight in kilograms by their height in meters squared ($kg/m^2$). The World Health Organization (WHO) defines an individual as overweight with a BMI ≥ 25 and as obese with a BMI value of ≥ 30 (WHO, 2013). Different classifications of obesity are also based on BMI values, wherein classes I, II, and III are defined by BMI ranges of 30.0-34.9, 35.0-39.9, and ≥ 40 respectively (Katzmarzyk & Mason, 2006). While BMI provides a quick and useful population-level measurement of overweight and obese status, the values should be used with caution since it may not correspond to the same degree of adiposity in different individuals (WHO, 2013). Other measures to define an individual as obese include calculating one's waist-to-hip ratio, caliper-measured skin

fold test, dual-energy X-ray absorptiometry (DEXA) scan, bioimpedance analysis, hydrostatic weighing, and clinical approaches defined by physicians classifying patients into specific categories based on both their BMI and state of health (Moon et al., 2011; Padwal, Pajewski, Allison, & Sharma, 2011; Shields et al., 2010; Yu, Rhee, Park, & Cha, 2010).

Multiple biological, psychological, and social factors contribute to the complexity of obesity, making it difficult to develop practical population-wide solutions. Although genetic, maternal, and perinatal aspects influence body fat content, one important contributing factor includes the imbalance between increased energy intake and decreased energy expenditure (Ahima, 2011; D. C. Lau et al., 2007; Shields et al., 2010; WHO, 2013). A significant shift in diet towards energy-dense foods and sedentary lifestyle with increasing global urbanization promotes an "obesogenic" environment, driving increased prevalence (Ahima, 2011; X. C. Lau, Chong, Poh, & Ismail, 2013). Although lifestyle modification is easily prescribed, treatment strategies focusing on diet and exercise are not effective for long-term weight loss and maintenance due to poor adherence and modest outcomes (D. C. Lau et al., 2007). Therefore, there is an urgent need to understand the pathophysiological processes involved in the manifestation of weight gain and obesity, which will allow for the discovery of effective therapeutics and preventative strategies aimed at conquering this disease state.

## 1.2 ADIPOSE TISSUE

### 1.2.1 Alterations of Adipose Tissue in Obesity

Body fat mass is determined by adipocyte number and size. As adiposity increases, it results in distinctive changes in white adipose tissue. Excessive accumulation of adipose tissue occurs due to significant adipose mass expansion primarily through adipocyte hypertrophy (increased fat cell volume) (Spalding et al., 2008). However, conflicting reports have yet to determine whether altered adipocyte hyperplasia or cell turnover significantly contributes to the manifestation of obesity (Arner et al., 2011; Cinti et al., 2005; Fujiwara et al., 2012; Spalding et al., 2008). Interestingly, reports have shown that adipose tissue expansion is not always pathological since “metabolically healthy obese” individuals have preserved insulin sensitivity, reduced cardiovascular risk, and lower expression of inflammatory markers, which suggests a basis for “healthy” adipose tissue expansion (Apovian et al., 2008; Peppa et al., 2012). Conversely, adipose tissue dysfunction predominately occurs in obesity and is characterized as an insulin resistant proinflammatory state due to pathological adipose tissue expansion, specifically in the visceral cavity (Hotamisligil, Shargill, & Spiegelman, 1993; Samaras, Botelho, Chisholm, & Lord, 2010). This state is characterized by extracellular matrix overproduction, reduced angiogenic remodeling and adipocyte hypertrophy leading to hypoxia-induced fibrosis, and increased infiltration of immune cells, subsequently creating a proinflammatory environment in many obese

fat tissues (Khan et al., 2009; Michailidou et al., 2012; Rausch, Weisberg, Vardhana, & Tortoriello, 2008).

Adipose tissue is a major contributor to the systemic chronic low-grade subacute inflammation described in obesity. Reports suggest that this state of inflammation contributes to many of the systemic pathological metabolic consequences related to obesity. Therefore, understanding the inflammatory process associated with obesity may result in key therapeutic strategies aimed at combating obesity and its associated chronic conditions.

### 1.2.2 Heterogeneity of Adipose Tissue – Housing Immune Inflammatory Players

Obesity can lead to altered cellular composition, phenotype, and activity in adipose tissue, which contributes to obesity-linked inflammation. This heterogeneous tissue is comprised of mature adipocytes, preadipocytes, fibroblasts, mesenchymal stem cells, endothelial cells, immune cells, vascular cells, and muscle cells (Ouchi, Parker, Lugus, & Walsh, 2011). Resident adipose tissue macrophages (ATMs) are most abundant in visceral fat and display notable diversity in their activities and functions (Bruun, Lihn, Pedersen, & Richelsen, 2005). During obesity development, ATMs undergo “phenotypic switching” where cells are transformed from an anti-inflammatory “alternatively activated” M2 form, seen primarily in lean adipose tissue, to a proinflammatory “classically activated” M1 form contributing to the obese inflammation. These polarized states are characterized by the expression of surface antigens, cytokines, and enzymes

where F4/80, macrophage galactose N-acetylgalactosamine lectin (cluster of differentiation (CD)301), interleukin (IL)-10, and arginase 1 are features of M2 ATMs, while expression of F4/80, CD11c, tumor necrosis factor (TNF)-α, IL-6, inducible nitric oxide synthase (iNOS), and C-C chemokine receptor (CCR) type 2 are of M1 ATMs (Lumeng, Bodzin, & Saltiel, 2007). M1 proinflammatory ATMs positively correlate with whole body insulin resistance, while a profound reduction in this population has been shown to improve insulin sensitivity (Di Gregorio et al., 2005; Patsouris et al., 2008). A substantial increase in ATM infiltration occurs in diet induced obesity, where this population can rise from 5-10% in lean adipose tissue to 60% in obese adipose tissue, and is positively correlated with both adipocyte size, body mass, and adiposity (Bruun et al., 2005; Weisberg et al., 2003). This suggests that immune cells have the ability to influence the metabolic health of individuals through alterations in their activity.

Shifts in adipose tissue T-cell subsets may promote macrophage polarization and recruitment. Lean adipose tissue is enriched with anti-inflammatory $CD4^+Foxp3^+$ regulatory T cells (Tregs), which reduce adipocyte inflammatory cytokine expression through the production of IL-10. Additionally, $CD4^+$ $T_H2$ cells, unique helper lymphocytes and eosinophils, promote M2 polarization through IL-4 and/or IL-13 production. An obese state however, downregulates $CD4^+$ $T_H2$, eosinophils, and Tregs (Feuerer et al., 2009; Moro et al., 2010; Odegaard et al., 2007; Winer et al., 2009; Wu et al., 2011). With obesity development, studies have observed an increase in the ratio of $CD8^+$ to $CD4^+$ T

cells preceding ATM accumulation. Interactions with obese adipose tissue resulted in $CD8^+$ T cell proliferation and activation, leading to recruitment, enhanced differentiation, and activity of macrophages, promoting an M1 phenotype (Nishimura et al., 2009). Of the $CD4^+$ T cells present, accumulating adiposity enhanced the recruitment and expansion of $CD4^+$ $T_H1$ interferon (IFN)-γ producing cells, further promoting the inflammatory M1 macrophage phenotype (Winer et al., 2009). Altogether, these studies suggest that macrophage recruitment and activation are influenced by $T_H1$ and $T_H2$-type signals, which potentially dictates the inflammatory status in adipose tissue.

### 1.2.3 Adipose Tissue - a Key Endocrine Organ of Inflammatory Mediators

Adipose tissue has long been viewed simply as a long term storage site for lipids. In the past twenty years, research findings have challenged this notion with the discovery of adipose tissue's endocrine ability to secrete numerous proteins termed adipokines. These bioactive substances can exert either deleterious or beneficial local and/or systemic effects due to their pro or anti-inflammatory properties. In obesity, excess adiposity dysregulates the expression of these factors, promoting pro-inflammatory responses that contribute to the development of obesity-linked complications. Although various factors have been discovered in adipose tissue, the classical adipokines primarily examined in obesity include leptin, TNF-α, and adiponectin (Ouchi et al., 2011).

Leptin was the first adipose specific adipokine identified for its role in regulating feeding behaviour and energy expenditure (Y. Zhang et al., 1994). As a satiety factor, leptin deficient (ob/ob) mice are hyperphagic, obese, and insulin resistant; however, leptin administration reversed these metabolic abnormalities (Muzzin, Eisensmith, Copeland, & Woo, 1996; Pelleymounter et al., 1995). Lean wildtype leptin treated mice had increased basal metabolic rate and decreased food intake, leading to fat-specific weight loss (Halaas et al., 1997). Lastly, leptin administration to lipoatrophic mice improved insulin sensitivity and hyperlipidaemia (Shimomura, Hammer, Ikemoto, Brown, & Goldstein, 1999). Despite favorable metabolic improvement with treatment, blood leptin levels positively correlate with adipose mass, suggesting obese individuals may experience leptin resistance since high circulating levels do not induce anorexic responses (Considine et al., 1996).

The leptin receptor is widely distributed, located in the central nervous system and hematopoietic cells, suggesting it may play a role in initiating or modulating immune responses (Lord et al., 1998; Tartaglia et al., 1995). This adipokine also has structural similarity to the helical cytokine family, which include growth hormone and IL-2 (Hill, Morea, & Chothia, 2002; F. Zhang et al., 1997). Studies have examined its pro-inflammatory properties using murine and human myeloid cells. *In vitro*, leptin stimulated human monocytes to proliferate and produce TNF-α and IL-6 in a dose-dependent manner, as well as increased the expression of the early activation marker

CD69 (Santos-Alvarez, Goberna, & Sanchez-Margalet, 1999). Using the murine J774A.1 macrophage cell line, leptin enhanced the expression of CC-chemokine ligands (CCL) CCL3, CCL4, and CCL5 through activation of the JAK2 (Janus kinase) and STAT3 (signal-transducer and activator of transcription) pathway, potentially promoting immune cell migratory responses (Kiguchi, Maeda, Kobayashi, Fukazawa, & Kishioka, 2009). Rodents treated with pro-inflammatory stimuli such as TNF-α and lipopolysaccharide (LPS) increased the expression and production of leptin in adipose tissue and in circulation, respectively (Grunfeld et al., 1996). Finally, leptin increased production of $T_H$-1 type cytokines, such as IL-2 and IFN-γ, while suppressing the $T_H$-2 cytokine IL-4 by T-cells or mononuclear cells stimulated *ex vivo* (Lord et al., 1998). Altogether, these results demonstrate that adipose derived factors can induce immune cell responses.

Tumor necrosis factor-α is primarily known for its role in inflammatory and autoimmune disease processes and is mainly expressed by monocytes and macrophages. TNF-α was the first proinflammatory adipokine identified that linked inflammation to obesity and insulin resistance. Various animal models of obesity and type 2 diabetes displayed increased adipose tissue TNF-α expression compared to lean controls (Hotamisligil et al., 1993). TNF-α promotes insulin resistance in muscle and adipose tissues by attenuating tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS1) (Hotamisligil, Budavari, Murray, & Spiegelman, 1994). Using recombinant soluble TNF-α receptor immunoglobulin G, neutralization of TNF-

induced signaling led to improved insulin sensitivity, with enhanced insulin signaling at these tissue sites without promoting weight loss (Hotamisligil et al., 1993). TNF-α expression in adipose tissue and in the circulation positively correlated with body weight and fat mass in human subjects, while weight loss reduced its expression (Kern et al., 1995; Ziccardi et al., 2002). Treating patients with severe autoimmune diseases such as rheumatoid arthritis and psoriasis, as well as obese subjects with TNF-inhibitors infliximab or etanercept, significantly improved insulin sensitivity, suggesting targeting inflammatory factors may exert beneficial therapeutic effects on obesity linked complications (Gonzalez-Gay et al., 2006; Marra et al., 2007; Stanley et al., 2011).

Adipose tissue's primary anti-inflammatory factor, adiponectin, is mainly produced by adipocytes, accounts for 0.01% of total plasma protein, and its circulating levels negatively correlate with visceral fat accumulation and risk of type 2 diabetes (Arita et al., 1999; Li, Shin, Ding, & van Dam, 2009; K. Maeda et al., 1996; Ryo et al., 2004). Adiponectin is a 244 amino acid polypeptide, containing a collagen-like domain, followed by a globular domain that shares sequence homology to collagens VIII and X and complement factor C1q (K. Maeda et al., 1996). Studies have shown that adiponectin production is significantly decreased in response to pro-inflammatory stimuli or metabolic stressors, such as TNF-α, hypoxia, and oxidative stress, which are upregulated in dysfunctional obese adipose tissue (Hosogai et al., 2007; Wang, Jenkins, & Trayhurn, 2005). Adiponectin deficient mice have elevated adipose tissue TNF-α

expression and severe diet induced insulin resistance, however, adiponectin administration restored these parameters to comparable levels in wildtype animals (N. Maeda et al., 2002). Overexpression of adiponectin in leptin deficient ob/ob mice improved systemic insulin sensitivity and reduced adipose tissue macrophage number and TNF-α expression (Kim et al., 2007). Altogether, these studies suggest that adiponectin may reverse metabolic dysfunction through the suppression of pro-inflammatory cytokine production.

Studies have also examined adiponectin's role in controlling inflammation through the modulation of macrophage function and phenotype. Adiponectin treatment of human monocyte-derived macrophages reduced intracellular cholesteryl ester content through suppression of class A scavenger receptor expression, leading to suppressed macrophage transformations into atherosclerotic foam cells (Ouchi et al., 2001). Also, treatment with this adipokine suppressed macrophage phagocytosis and LPS-stimulated TNF-α production, while stimulating production of the anti-inflammatory cytokine IL-10 *in vitro* (Kumada et al., 2004; Yokota et al., 2000). Isolated peritoneal and adipose tissue macrophages from adiponectin deficient animals had increased proinflammatory M1 expression markers with decreased M2 markers, however, systemic delivery of this adipokine stimulated the expression of the anti-inflammatory M2 marker arginase 1. Lastly, adiponectin treatment of human monocyte-derived and adipose tissue macrophages stimulated the expression of M2 markers, while attenuating

M1 markers (Ohashi et al., 2010). Overall, these findings suggest adiponectin, an adipose-derived factor, may reduce inflammation through the modulation of systemic and local macrophage function and phenotype.

Thorough assessment of the intricate interactions between inflammatory immune mediators and adipocytes throughout the development of obesity is critical to sufficiently evaluate the role of inflammation in adiposity. Evidence of bidirectional interactions between immune cells and adipocytes has been described. For example, Tregs and M1 macrophages directly affect adipocyte glucose utilization by modulating the expression of glucose transporters, suggesting immune cells can affect metabolic parameters in fat cells (Feuerer et al., 2009; Lumeng, Deyoung, & Saltiel, 2007). As well, signaling molecules, such as cytokines, can influence both immune and adipocyte metabolic responses, and have been linked to obesity development. Interleukin-15 (IL-15) is an immunomodulatory proinflammatory cytokine that has been studied primarily for its effects on both innate and adaptive immunity. Interestingly, it has also been shown to affect lean/fat body composition (Carbo et al., 2001). We and others have demonstrated that lower circulating IL-15 levels are associated with obesity and negatively correlate to BMI, trunk fat, and total fat mass in human subjects (Barra et al., 2009; Nielsen et al., 2008). Therefore, understanding how IL-15 affects adipose tissue mass will aid in examining the role of inflammatory factors in body weight regulation.

## 1.3 INTERLEUKIN-15

### 1.3.1 The Molecular Characteristics and Expression of Interleukin-15

Interleukin-15 (IL-15) is a 14-kDa glycoprotein encoded by a 34kb region on the human chromosome 4q31 and murine chromosome 8. Human IL-15 shares 73% sequence homology at the nucleotide and amino acid level with murine IL-15 (Anderson et al., 1995; Grabstein et al., 1994). Its characteristic two disulfide crosslinks at cysteine (Cys)35-Cys85 and Cys42-Cys88 and helical crystal structure classify IL-15 as a member of the four α-helix bundle family of cytokines (Grabstein et al., 1994; Ring et al., 2012). Throughout the body, IL-15 messenger ribonucleic acid (mRNA) is expressed in several tissues including skeletal muscle, placenta, liver, heart, lung, kidney, macrophage/monocytes, dendritic cells (DCs), fibroblasts, and epithelial cells (Doherty, Seder, & Sher, 1996; Grabstein et al., 1994; Mattei, Schiavoni, Belardelli, & Tough, 2001).

Despite widespread mRNA expression, protein production is primarily limited to monocytes/macrophages and DCs, indicating that IL-15 is predominately post-transcriptionally regulated (Steel, Waldmann, & Morris, 2012). Two forms of mature IL-15 mRNA exist, differing in their signal peptide lengths. The long signal peptide (LSP) IL-15 isoform is 48 amino acids long and encoded within exons 3-5, whereas the short signal peptide (SSP) is 21 amino acids long and encoded by exons 4A-5. Despite identical protein products, each isoform has distinct intracellular trafficking, where the LSP-IL-15 targets the Golgi apparatus, early endosomes, and the endoplasmic reticulum (ER)

secretory pathway, whereas the non-secreted SSP-IL-15 is restricted to the cytoplasm and nucleus (Tagaya et al., 1997). Furthermore, secreted LSP-IL-15 is either released by the cell or remains membrane bound.

### 1.3.2 Interleukin-15 Signalling

IL-15 signals through a heterotrimeric receptor complex, including a β subunit shared with IL-2 and a common gamma chain ($\gamma_c$) subunit shared with IL-2, -4, -7, -9, and -21. The third unique IL-15-specific α subunit confers high affinity binding between IL-15 and the complex by substantially increasing the affinity of IL-15 to the β-subunit (Giri et al., 1995; Ring et al., 2012). The human IL-15 receptor-α (IL-15Rα) gene is encoded on chromosome 10, comprising of seven exons, which produces eight different isoforms due to alternative splicing (Dubois et al., 1999). This receptor is widely expressed, binds to IL-15 with high affinity ($K_d$>$10^{-11}$M), and can interact with IL-15 intracellularly as a complex in cells expressing both the cytokine and the α-subunit prior to its secretion. This receptor subunit is critical to the production and emergence of IL-15 from a cell, since DCs from IL-15R$\alpha^{-/-}$ mice cannot secrete IL-15 (Mortier, Woo, Advincula, Gozalo, & Ma, 2008). Moreover, co-transfected human 293 cells with IL-15Rα and IL-15 plasmids increased detectable IL-15 in supernatants compared to cells transfected with IL-15 plasmid alone. In parallel, co-delivery of injected IL-15Rα and IL-15 plasmids *in vivo* significantly increased IL-15 serum level and bioactivity (Bergamaschi et al., 2008). Binding to this receptor subunit may also increase the half-life of IL-15 by preventing

proteolytic degradation (Bergamaschi et al., 2009). Furthermore, a recent study showed that virtually all detectable plasma IL-15 was associated with IL-15Rα in lymphodepleted melanoma patients and mice (Bergamaschi et al., 2012). Lastly, it has been observed that once bound intracellularly, IL-15Rα may retain IL-15 on its cell surface to induce trans-signalling (Mortier et al., 2008).

Trans-presentation is the primary mode by which IL-15 signals *in vivo* wherein IL-15Rα binds to IL-15 intracellularly, is expressed as a complex on the cell surface (typically of a myeloid cell), and then presented to an adjacent cell expressing surface receptor β and $\gamma_c$ subunits (Burkett et al., 2004; Castillo, Stonier, Frasca, & Schluns, 2009; Mortier et al., 2008). This then activates the JAK/STAT signalling pathway, leading to phosphorylation and activation of JAKs-1/3 and STATs-3/5. The β and $\gamma_c$ subunits are associated with JAK-1/STAT3 and JAK-3/STAT5 activation, respectively, and are critical for initiating its immunological effects (Johnston et al., 1995).

### 1.3.3 Immunological Effects of Interleukin-15

Interleukin-15 was first described for its ability to enhance anti-tumor responses and stimulate T-lymphocyte proliferation (Burton et al., 1994; Grabstein et al., 1994). Since then, this pleiotropic cytokine has been observed to have important roles in both innate and adaptive immune responses. In innate immunity, IL-15 primarily promotes the development, survival, and activation of natural killer (NK) cells. Activated NK cells mediate cytotoxic effects through antibody dependent cellular cytotoxicity, cytokine

production (IFN-γ and TNF-α), granzyme/perforin release, and expression of death receptor ligands (Srivastava, Lundqvist, & Childs, 2008). In adaptive immunity, IL-15 primarily stimulates the proliferation of $CD4^+$ and $CD8^+$ T cells, and facilitates the induction of cytotoxic T-lymphocytes through the production of IFN-γ, TNF-α, and perforin/granzyme (Waldmann & Tagaya, 1999). IL-15 also controls the survival and proliferation of the memory $CD8^+CD44^{hi}$ T cell subset (Kanegane & Tosato, 1996). In murine models, overexpression of IL-15 (IL-15tg) results in a substantial increase in NK and $CD8^+CD44^{hi}$ memory T cells, while IL-15 deficient animals (IL-15-/-) lack NK cells and have fewer $CD8^+$ T cells (Fehniger, Suzuki, Ponnappan et al., 2001; Kennedy et al., 2000). In B lymphocytes, IL-15 induces proliferation, differentiation, and increases immunoglobulin secretion (Armitage, Macduff, Eisenman, Paxton, & Grabstein, 1995).

IL-15 can also stimulate antigen presenting cells directly and indirectly through NK cell mediated IFN-γ release. DCs incubated with IL-15 are resistant to apoptosis, enhance IFN-γ secretion and expression of co-stimulatory molecules, and promote maturation through increased expression of CD83, CD86, CD40, and major histocompatibility complex (MHC) class II (Anguille et al., 2009). In macrophages, IL-15 increases phagocytosis, expression of IL-12 and monocyte chemoattractant protein-1 (MCP-1), and secretion of IL-6, IL-8, and TNF-α (Budagian, Bulanova, Paus, & Bulfone-Paus, 2006). Macrophages exposed to IL-15 overexpression *in vivo* produce higher levels of nitric oxide (Davies, Reid, Medina, Lichty, & Ashkar, 2008). Altogether, these results

demonstrate the vast effects IL-15 has on various immune cell populations. Interestingly, this cytokine has garnered great excitement for its potential ability to directly influence metabolic tissues such as adipose tissue.

### 1.3.4 Interleukin-15's Effects on Adipose Tissue

The possible effects of IL-15 on adipose tissue were first described by a research group from the Universitat de Barcelona. While determining the possible antagonizing catabolic skeletal muscle effects of IL-15 in a rodent cachexic model, investigators observed cytokine treatment in healthy rats significantly reduced white adipose tissue without affecting food intake (Carbo et al., 2000). Upon further investigation, this group showed that seven consecutive days of IL-15 treatment *in vivo* significantly reduced lipoprotein lipase (LPL) activity and lipogenesis in white adipose tissue, as well as circulating total and very low density lipoprotein (VLDL) triglycerides (Carbo et al., 2001). Reduced LPL activity and white adipose tissue mass was also seen in IL-15 treated leptin deficient obese ob/ob mice (Alvarez et al., 2002). In genetically modified mice, IL-15 overexpression in skeletal muscle, resulted in elevated cytokine secretion into circulation, and reduced fat mass and body weight in 28 week old male mice fed either a low or high fat/energy diet (Quinn, Anderson, Strait-Bodey, Stroud, & Argiles, 2009). These observational studies suggest that IL-15 may play a role in body composition by affecting adipose tissue mass.

*In vitro* studies have demonstrated that IL-15 can directly impact cultured adipocytes. We and others have shown that IL-15 treatment inhibited lipid accumulation in differentiating 3T3-L1 preadipocytes and adipose derived human adipocytes (Almendro et al., 2009; Barra et al., 2009). Recombinant IL-15 protein also stimulated the secretion of the anti-inflammatory adipokine adiponectin by 3T3-L1 adipocytes in a dose and time dependent manner (Quinn, Strait-Bodey, Anderson, Argiles, & Havel, 2005). The molecular pathways mediating these effects have not been well characterized. Using ribonuclease protection assays, mRNA expression of IL-15Rα, IL-2/15Rβ, and $\gamma_c$ subunits in rodent white adipose tissue suggests that IL-15 may directly signal in adipocytes, affect white adipose tissue mass, and possibly function as an anti-obesogenic factor (Alvarez et al., 2002). IL-15 treatment induces protein expression of phosphorylated STAT-5 and stress-activated protein kinase/c-Jun NH2-terminal kinases (SAPK/JNK) 54 and SAPK/JNK46 in mature adipocytes; however, the effect(s) of activating these signaling pathways in adipocytes were not examined by the authors (Fuster et al., 2011). Conflicting reports demonstrate it is not yet determined whether or not IL-15 can directly stimulate lipolysis in cultured adipocytes (Fuster et al., 2011; Quinn et al., 2005). Altogether, these studies suggest that IL-15 may mediate its effects directly on adipocytes.

Despite these observations, further evidence demonstrating whether varying IL-15 expression can modulate body weight and adipose tissue, as well as understanding

the underlying mechanisms mediating this phenomenon is essential. A thorough investigation examining possible factor(s) are needed using both *in vivo* and *in vitro* approaches.

Deficiencies in other factors involved in immunity have been shown to indirectly affect body weight. For example, toll-like receptor 5 deficient animals (TLR5-/-) exhibit hyperphagia, increased adiposity, insulin resistance, and hyperlipidemia compared to control animals. These significant metabolic characteristics correlated with changes in the composition of gut bacteria. Interestingly, these metabolic abnormalities were transferrable through colonization of wild-type germ free recipients with TLR5-/- donor gut bacteria (Vijay-Kumar et al., 2010). Similarly, mice deficient in lymphotoxin, a factor involved in gut immunity, were protected against diet induced obesity. Gut bacterial transplantation from lymphotoxin deficient donor animals conferred leanness to germ-free recipients (Upadhyay et al., 2012). These results suggest that factors involved in immunity may also indirectly influence adiposity and body weight by modulating mediators involved in weight regulation such as the gut microbiota. Since IL-15 administration resulted in a significant reduction in intestinal triglyercol absorption in rodents, and the gut bacterial composition influences macronutrient absorption, it is possible that IL-15 mediates its effects on body weight and adiposity through variation in the gut microbiota (Almendro et al., 2005; Backhed et al., 2004; Hooper et al., 2001).

## 1.4 GUT MICROBIOTA

### 1.4.1 The Complexity of the Gut Microbiota

The human intestine has coevolved with a vast number and diversity of microorganisms, collectively referred to as the gut microbiota, which contribute to a diverse set of functions (Ley, Hamady et al., 2008; Ley, Lozupone, Hamady, Knight, & Gordon, 2008). The microbiota refers to the complete microbial community inhabiting a specific environment. Cellular density along the length of the gut increases substantially, where the colonic microbiota is characterized as the densest and most diverse microbial community in the gut and entire human body (Tremaroli & Backhed, 2012). Conservative estimates using culture independent methods including 16S ribosomal RNA profiling and direct sequencing approximate $10^{14}$ bacteria and archaea inhabit the gut, consisting of 1000 species, primarily dominated by anaerobic bacteria (Qin et al., 2010). Approximately $10^{3}$-$10^{5}$ bacteria are found in the proximal portion of the small intestine (duodenum and jejunum) and are predominately aerobic, while there are $10^{10}$-$10^{11}$ organisms in the colon which are generally anaerobic. The terminal ileum acts as a transition zone, containing relatively equal proportions of aerobic and anaerobic species (Berg, 1996). This complex community is dominated by bacterial species (~90%) belonging to the Firmicutes and Bacteroidetes phyla in healthy individuals (Eckburg et al., 2005). Although each person has a distinct, highly variable microbiota, individuals in a specific geographical region and family members share a set of conserved gut

colonizers and genes comprising an identifiable 'core microbiota' (Qin et al., 2010; Turnbaugh, Hamady et al., 2009).

The collective genome of the gut microbiota, referred to as the microbiome, initiates many unique metabolic functions not encoded by the human genome. In fact, this microbial gene set is approximately 150-times larger than the human complement, enabling host-microbial interactions and mutualism (Qin et al., 2010). Metabolic functions of gut bacteria include the production of some essential vitamins such as vitamin K, folic acid, and vitamin B12, intestinal bile acid metabolism and recirculation, and the processing of otherwise indigestible dietary components such as plant polysaccharides into simple sugars, short-chain fatty acids (SCFAs), and other nutrients (Mai & Draganov, 2009). Compelling evidence demonstrates substantial alterations in the composition and metabolic function of the gut microbiota are associated with weight gain and increased host adiposity. These findings support the notion that the gut microbiome together with host genotype and lifestyle may contribute to the development of obesity and its comorbidities.

### 1.4.2 Association Between the Gut Microbiota and Obesity

Landmark studies originating from Dr. Jeffrey Gordon's laboratory at Washington University first demonstrated the potential link between the intestinal microbiota and host adiposity using germ free mice, obese animal models, and human subjects. Germ free animals, specifically mice, are a powerful tool when examining the effects of the gut

microbiota on host physiology. These animals are born and reared without exposure to any live microbes under axenic conditions. Bacterial colonization in these mice with either selected microbial species or whole communities from mice or humans can allow for the examination of the transmissibility of physiological and pathological phenotypes, and determination of its role in promoting these characteristics (Tremaroli & Backhed, 2012). This group showed conventionally raised mice had 42% more total body fat, despite consuming significantly less food, than germ free mice. Colonization of germ free recipients with "normal" gut microbiota from conventionally raised animals, resulted in a 60% increase in total body fat and adipocyte hypertrophy despite decreased food intake (Backhed et al., 2004). Germ free mice were also protected against diet induced obesity unlike conventional mice (Backhed, Manchester, Semenkovich, & Gordon, 2007). Lastly, similar results were obtained when gut microbiota transplants from diet induced obese mice into germ free recipients resulted in significantly more total fat mass gain than transplants from lean donors (Turnbaugh, Backhed, Fulton, & Gordon, 2008).

Obesity and changes in body weight can alter the composition of the gut microbiota. In genetically obese hyperphagic ob/ob mice, the caecal microbiota contained 50% fewer Bacteroidetes and a proportional division-wide increase in Firmicutes compared to lean littermates independent of food consumption (Ley et al., 2005). Similar findings were detected in the fecal microbiota of lean versus obese human subjects, revealing reduced gut bacterial diversity (Ley, Turnbaugh, Klein, &

Gordon, 2006; Turnbaugh, Hamady et al., 2009). In diet induced obese animals, a similar reduction in Bacteriodetes and a proportional increase in Firmicutes was observed; however, a bloom of a single Mollicutes class within the Fimicutes was seen, leading to a dramatic fall in overall diversity (Turnbaugh et al., 2008). This dramatic shift in the gut microbiota phyla is rapid, occurring within 24 hours of diet change from a low-fat, plant polysaccharide-rich diet to a 'Western' high fat, high sugar diet (Turnbaugh, Ridaura et al., 2009). Weight reduction intervention strategies with fat/carbohydrate low calorie restricted diets or Roux-en-Y gastric bypass surgery positively correlated with increased Bacteriodetes and decreased Firmicutes levels division-wide (Furet et al., 2010; Ley et al., 2006). Although obesity and energy intake can affect its microbial composition, the functional complexity of the gut microbiota has made it difficult to establish the underlying mechanisms of the gut microbiome in promoting obesity; however, functional connections in both animal and human studies have revealed its effects on host metabolism.

### 1.4.3 Uncovering Mechanisms of the Gut Microbiota on Host Metabolism

#### 1.4.3.1 'Mining' for Calories –Energy Extraction by the Gut Microbiota

Carbohydrates are an important energy source for humans and microbial cells, but enzymes encoded in the human genome cannot degrade most complex carbohydrates and plant polysaccharides. Non-digestible carbohydrates such as cellulose, resistant starch, inulin, and xylans undergo enzymatic breakdown by

microbially produced enzymes, such as glycoside hydrolyases and polysaccharide lyases, and ferment in the colon (Sonnenburg et al., 2005; Tremaroli & Backhed, 2012; Xu et al., 2003). This process yields end products used in microbial growth and SCFAs such as acetate, propionate, and butyrate that provide energy for colonic epithelial cells or act as gluconeogenic and lipogenic substrates in peripheral tissues once absorbed (Backhed et al., 2004; Bergman, 1990). Colonization of germ free mice with gut bacteria from conventionalized animals resulted in increased mRNA expression of Na+/glucose cotransporter (SGLT-1), and intestinal monosaccharide absorption, leading to *de novo* hepatic lipogenesis (Backhed et al., 2004; Hooper et al., 2001). Promoting efficient carbohydrate fermentation with colonization of certain bacterial species leads to increased energy absorption from the gut and host adiposity, suggesting gut bacterial composition plays a role in nutrient digestion and absorption (Samuel & Gordon, 2006). The microbiome from diet induced obese mice were enriched with phosphotransferase proteins involved in importing simple sugars, β-fructosidase capable of degrading fructose-containing carbohydrates into SCFAs, fermentation, and depleted of genes associated with motility (Turnbaugh et al., 2008). Altogether these findings suggest the diet induced obese gut microbiome may increase gut transit time to permit the import and fermentation of carbohydrates into SCFAs and which are subsequently absorbed by the host.

Compared to conventionally raised animals, germ free rodents have reduced intestinal levels of SCFAs (Hoverstad & Midtvedt, 1986) and two times more urinary and fecal caloric output when fed a polysaccharide-rich diet (Wostmann, Larkin, Moriarty, & Bruckner-Kardoss, 1983). Similarly, ob/ob mice have increased cecal SCFAs, reduced fecal energy content, and a plethora of genes related to dietary polysaccharide degradation and fermentation in the gut microbiome compared to lean wildtype animals. These traits were also transferable to germ free recipients colonized with ob/ob gut microbiota. These mice also harbor more methanogenic Archaea, which increase bacterial fermentation efficiency (Turnbaugh et al., 2006). Fecal samples in human obese subjects also showed higher representation of methanogenic Archaea (H. Zhang et al., 2009), and a microbiome enriched for carbohydrate processing (Turnbaugh, Hamady et al., 2009). Altogether, these results suggest that altered fermentation of complex carbohydrates and an increased capacity to harvest energy in the obese gut microbiota lead to increased availability of nutrients resulting in increased host adiposity.

1.4.3.2 Modulation of Host Metabolic Signaling by the Gut Microbiota

Studies show the gut microbiota and its products can directly regulate host gene expression, leading to increased body weight and host adiposity through three different mechanisms. Fasting-induced adipose factor (Fiaf), also known as angiopoetin-like protein 4, is a circulating LPL inhibitor, expressed by the intestine, liver and adipose tissue, which prevents cellular uptake of fatty acids and adipocyte triglyceride

accumulation (Yoon et al., 2000). Conventionalization of germ free animals suppressed intestinal Fiaf expression, relieving inhibition of LPL, increasing fatty acid uptake. Lastly, germ free Fiaf-/- animals are not protected against diet induced obesity compared to wildtype germ free mice, suggesting the gut microbiota regulates peripheral host fat storage through the expression of Fiaf (Backhed et al., 2004).

Adenosine monophosphate (AMP)–activated protein kinase K (AMPK) is an enzyme activated by metabolic stresses resulting in an increased ratio of intracellular AMP to adenosine triphosphate (ATP) (Kahn, Alquier, Carling, & Hardie, 2005). Phosphorylation of AMPK stimulates fatty acid oxidation (FAO) in target tissues. Despite high caloric intake, the persistent leanness of germ free animals is associated with increased activity of AMPK in both the liver and skeletal muscle, suggesting the gut microbiota may suppress FAO through AMPK (Backhed et al., 2007). Lastly, SCFAs synthesized from fermented complex dietary carbohydrates can act as signaling molecules and bind to G-protein-coupled receptors (GPCRs) expressed by gut epithelial cells. Proprionate and acetate are ligands for GPCRs Gpr41 and Gpr 43 (Brown et al., 2003; Le Poul et al., 2003). Gpr41-/- mice have decreased expression of the enteroendocrine hormone peptide YY, which is involved in gut motility. Decreased peptide YY levels in Grp41-/- mice leads to faster intestinal transit and increased fecal SCFAs compared to wildtype controls, suggesting the gut microbiota can affect intestinal motility and the ability to harvest energy (Samuel et al., 2008).

1.4.3.3 Impact of Gut Bacteria on Endotoxemia, Inflammation, and Weight Gain

Obesity is an inflammatory condition characterized by altered immune cell and adipokine activity and profile, resulting in systemic and adipose tissue inflammation. Elevated bacterial components peptidoglycan and endotoxin/LPS from the gut microbiota are also associated with obesity, and may promote inflammation at peripheral tissues. After 4 weeks, high fat fed mice have chronically elevated plasma LPS concentrations, increased LPS-expressing bacteria in the gut, and markers of adipose tissue inflammation compared to control animals (Cani et al., 2007). Human subjects fed a high fat or high carbohydrate diet over 3 days also had increased LPS plasma concentrations (Amar et al., 2008). In the absence of a high fat diet, mice infused with LPS subcutaneously had increased liver, adipose tissue, and whole body weight gain, along with fasting glucose and insulin levels comparable to high fat-fed mice; however, CD14-/- mice were unresponsive to LPS-induced metabolic dysfunction (Cani et al., 2007). Antibiotic treatment in high fat fed mice and ob/ob mice induced changes in gut microbial composition, reduced LPS plasma concentrations and cecal LPS content, as well as correlated with reduced weight gain (Cani et al., 2008). Altogether, these results suggest a potential link between endotoxemia from gut bacterial components, inflammation, and weight gain.

Obesity is associated with significant alterations in the composition and metabolic function of the gut microbiota through enhanced energy extraction and

modulation of host genes, directly affecting energy expenditure and storage. Colonization studies demonstrate the gut microbiota is a transmissible obesogenic trait using germ free animals, demonstrating its ability to promote host adiposity. Lastly, bacterial components released by the gut contribute to the pro-inflammatory environment associated with obesity, suggesting compromised gut integrity can lead to altered host adiposity and energy expenditure. Having said that, evaluating the role of the gut microbiota is critical when examining factors involved in modulating body weight and adiposity.

## 1.5 MITOCHONDRIAL DYSFUNCTION

Since obesity is defined as a state of abnormal or excessive adiposity, therapeutic strategies aimed at promoting the utilization of these bodily reserves have been examined. Mounting evidence indicates that mitochondrial failure to produce energy from nutrients is associated with obesity, its comorbities, and ectopic lipid deposition (Golbidi, Mesdaghinia, & Laher, 2012). Since the mitochondria are the major site of lipid catabolism and utilization, targeting factors that promote FAO may have therapeutic benefit.

Several studies have examined the effects of IL-15 on mitochondrial FAO in skeletal muscle. Using $^{14}C$ labeled lipids, one study showed that IL-15 administration resulted in a significant increase in whole body and skeletal muscle FAO, as well as

decreased muscle fat content in male Wistar rats. These results correlated with increased expression of the transcription factor peroxisome proliferator activated receptor (PPAR)-δ, which is involved in fatty acid catabolism (Almendro et al., 2006). Studies have also demonstrated that skeletal muscle from IL-15Rα-/- mice have increased mitochondrial density and deoxyribonucleic acid (DNA) content, and increased expression of PPAR-δ, mitochondrial complexes involved in the electron transport chain (ETC), and the mitochondrial biogenesis factor PPAR-γ coactivators (PGC)-1α (Pistilli et al., 2011; Pistilli, Guo, & Stauber, 2013). Similarly, transgenic mice overexpressing IL-15 in skeletal muscle have shown increased mRNA and protein expression of factors involved in promoting lipid oxidation in extensor digitorum longus muscles compared to control animals (Quinn, Anderson, Conner, & Wolden-Hanson, 2013). These results suggest that varying IL-15 and receptor subunit expression may affect the expression of mitochondrial markers involved in skeletal muscle FAO.

Cytokine treatment has also been shown to increase the expression of various mitochondrial markers involved in fatty acid transport and catabolism in brown adipose tissue such as carnitine palmitoyl transferases (CPT)-1 and acyl coenzyme A synthase 4, respectively (Almendro et al., 2008). However, it is not known whether IL-15 affects mitochondrial markers involved in FAO in white adipose tissue. Since increased IL-15 expression is associated with leanness and FAO in skeletal tissue, a thorough

examination of this organelle is required to determine whether altered mitochondrial function may contribute to IL-15's effects on adipose tissue.

### 1.5.1 Mitochondria – The Cell's 'Power House'

Mitochondria are enclosed double membrane organelles within nucleated cells that coordinate numerous metabolic reactions. The outer and inner mitochondrial membranes define two separate compartments – the matrix enclosed by the inner membrane, and the intermembrane space located between membranes. The primary function of mitochondria is to produce energy, in the form of ATP, which enables various essential cellular processes to transpire. Cellular ATP production involves the catabolism of fatty acids and glucose by beta oxidation and glycolysis, respectively. In circulation, triglycerides are carried to tissues by lipoproteins and hydrolyzed into free fatty acids (FFAs) and glycerol by capillary bound LPL upon cell entry. In the cytosol, FFAs react with coenzyme A (CoA), are converted into fatty acyl-CoA, and are subsequently shuttled into the mitochondria; however, very long chain FFAs (≥22 carbon atoms) are first oxidized in peroxisomes prior to entry. Short and medium chained fatty acids freely enter the mitochondrial matrix while long chained fatty acids (≥14 carbon atoms) are transported by the enzyme CPT1. Oxidation begins at the second carbon atom adjacent to the carboxyl group, where acyl-CoA dehydrogenase catalyzes the oxidation of the acyl group. Subsequently, the fatty acid undergoes hydration, a second oxidation step, and thiolysis, where a thiolase catalyzes the release of acetyl–CoA. This four step process is

repeated, removing two carbons each cycle, until the fatty acid is reduced to an acetyl-CoA molecule. This process also generates reducing agents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) described below. The end product of glycolysis, pyruvate, is also converted into acetyl-CoA molecules in the mitochondrial matrix, which then enter into the Krebs cycle (Gropper, Smith, & Groff, 2009).

As the initial intermediary metabolite of the Krebs cycle, acetyl-CoA undergoes additional enzymatically driven oxidative reactions, producing few ATP molecules, and transferring electrons to form reducing agents NADH and $FADH_2$. These reducing agents are also formed during beta oxidation and glycolysis. Through the process of oxidative phosphorylation (OXPHOS), NADH and $FADH_2$ relay electrons through a series of protein complexes comprising the electron transport chain (ETC), located on the inner mitochondrial membrane. Simultaneously, protons are pumped from the matrix into the intermembrane space, resulting in a proton/pH gradient across the inner membrane. This chemiosmotic gradient fuels the conversion of adenosine diphosphate (ADP) to ATP, where protons enter back into the matrix through the ATP synthase complex generating the majority of ATP as well as reactive oxygen species (ROS). In aerobic respiration, the breakdown of one glucose molecule generates 36-38 ATP molecules, whereas a 16 carbon saturated fatty acid, like palmitate, generates 129 (Marcovina et al., 2013; Pintus, Floris, & Rufini, 2012; Rogge, 2009). Compelling evidence demonstrates that

substantial reduction in mitochondrial function and activity are associated with metabolic disease and adiposity. These findings support the notion that mitochondrial dysfunction may contribute to the development of obesity and its comorbidities.

### 1.5.2 Association Between Dysfunctional Mitochondria and Obesity

The link between metabolic disease, such as obesity and type 2 diabetes, and mitochondrial dysfunction has been examined in obese animal models and human subjects. In human subjects, decreased mitochondrial content (measured by mitochondrial (mt) DNA copy number) in white adipocytes inversely correlated with insulin resistance and BMI. It has been hypothesized that significant reductions in adipose tissue mtDNA may lower the adipocyte's lipid storing capacity, since mtDNA content directly correlated with basal and insulin-induced lipogenesis. Therefore, this would promote ectopic lipid accumulation in peripheral tissues such as muscle and liver, and insulin resistance (Kaaman et al., 2007; Patti & Corvera, 2010). In parallel, down-regulation of nuclear-encoded OXPHOS gene expression in visceral adipose tissue of type 2 diabetic human subjects correlated with level of obesity (Dahlman et al., 2006).

Significant mitochondrial defects specific to white adipose tissue occur in both ob/ob and obese diabetic db/db mice compared to control mice, independent of defects in muscle and liver. Mitochondrial DNA content is significantly lowered in isolated adipocytes, suggesting significant mitochondrial loss specific to adipose tissue occurs in these animals. Abnormal mitochondrial morphology and functionality, measured by FAO

and respiration rates, were also reduced compared to isolated wildtype white adipocytes (Choo et al., 2006). Using microarray technology, genetic expression of mitochondrial host enzymes and transport proteins necessary for the Kreb's cycle, FAO, and OXPHOS pathways, all of which contribute to ATP production, were suppressed in adipose tissues of high fat fed and db/db mice compared to their respective control counterparts (Rong et al., 2007). Altogether, these results suggest mitochondrial dysfunction in adipose tissue occurs in obese murine models and human subjects.

### 1.5.3 Possible Mechanisms Involved in Promoting Mitochondrial Dysfunction

The mechanisms by which obesity mediates a reduction in adipose tissue mitochondrial content are unknown. Downregulation of mitochondrial biogenesis markers such as PGC-1α and/or PGC-1β have been observed in adipose tissue of obese individuals and murine models (Semple et al., 2004; Wilson-Fritch et al., 2004). Increased levels of ROS and oxidative stress have also been associated with obesity. Oxidative stress is the imbalance between ROS production and the impairment of antioxidant enzymatic defenses, resulting from excess production of free radicals and/or depletion of antioxidant defenses. This imbalance leads to oxidative damage of lipids, proteins, and DNA. A potent source of mitochondrial ROS comes from the ETC, primarily producing reduced oxygen $O_2^-$, hydrogen peroxide ($H_2O_2$), and hydroxyl radical $OH^-$ (Crujeiras, Diaz-Lagares, Carreira, Amil, & Casanueva, 2013; Patti & Corvera, 2010). The systemic oxidative stress biomarkers plasma thiobarbituric acid reactive substance

(TBARS) and urinary 8-epi-prostaglandin F2α were strongly correlated with visceral adiposity in obese subjects (Fujita, Nishizawa, Funahashi, Shimomura, & Shimabukuro, 2006; Furukawa et al., 2004).

Elevated production of TBARS, an oxidative marker of lipid peroxidation (LPO), and ROS $H_2O_2$ was observed in plasma and white adipose tissue of high fat fed and genetic murine models of obesity compared to respective controls. No significant differences were found in liver and skeletal muscle tissues. Results showed that activation of the NADPH oxidase complex, a major source of ROS, and decreased expression and activity of antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) contributed to oxidative stress specifically in accumulated white adipose tissue *in vivo* and cultured 3T3-L1 adipocytes. Increased ROS production and subsequent oxidative stress resulted in the expression and production of proinflammatory adipokines such as TNF-α, IL-6, and plasminogen activator inhibitor–1 (PAI-1), while suppressing the anti-inflammatory adipokine adiponectin. Without affecting body weight, inhibition of the NADPH oxidase complex had significant effects *in vivo*, including reducing ROS production and oxidative stress in white adipose tissue. This inhibition also upregulated adiponectin and downregulated TNF-α expression, decreased ectopic hepatic lipid deposition, and improved blood insulin, glucose, and triglyceride concentrations (Furukawa et al., 2004). Altogether, these results suggest that white adipose tissue is a major source of oxidative stress in obesity, through increased ROS

production and impaired antioxidant defenses. This imbalance in ROS production dysregulates adipokine expression contributing to the inflammatory state characteristic to obesity. Therefore, targeting factors that may promote mitochondrial lipid utilization, while inducing minimal damage and reducing adiposity in animal models of obesity may have therapeutic benefits.

## 1.6 RATIONALE, HYPOTHESIS, AND THESIS OBJECTIVES

Obesity is a major health concern worldwide and is associated with the development of numerous chronic diseases. A thorough understanding of the onset of obesity is critical, but complicated, since numerous factors contribute to the manifestation of this disease state. Recently, obesity has been described as a complex disease characterized by chronic low grade inflammation. Abnormal adipose tissue expansion is accompanied by an increased presence of proinflammatory immune cells, dysregulated adipokine expression, oxidative stress, and is associated with significant changes in the bacterial composition of the gut. Therefore, determining how immune modulators such as proinflammatory cytokines, like IL-15, influence body weight and fat mass is essential in examining the role of inflammation in the development of obesity.

While IL-15 has been studied extensively in activating immune responses, it also promotes decreased adiposity and is negatively associated with obesity (Barra et al., 2009). Given the importance of inflammatory factors in obesity, we hypothesized that

**varying levels of interleukin-15 cause significant differences in body weight and lipid deposition via a lymphocyte-independent mechanism.** The overall objective of this PhD thesis was to elucidate the role of IL-15 in modulating adipose tissue and its underlying mechanisms in the following aims:

1) To characterize the effect of varying IL-15 expression on body weight, adipose tissue mass, and its association with obesity.
2) To determine the role of lymphocytes in mediating IL-15 induced weight loss.
3) To elucidate the underlying mechanisms of varying IL-15 expression on body weight and adiposity described in aim 1.

Briefly, the experimental results presented in this thesis demonstrate that IL-15 is an important regulator of adipose tissue and body weight. These findings show the vast effects of IL-15 in modulating both the metabolic parameters and immune composition of adipose tissue, furthering our knowledge of IL-15 biology, which may contribute to novel therapeutic strategies for the treatment and prevention of obesity.

**CHAPTER 2**

# Interleukin-15 Contributes to the Regulation of Murine Adipose Tissue and Human Adipocytes

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This manuscript examines the effects of Interleukin-15 and its association with adiposity. This study demonstrates that over time, over expression of IL-15 in mice was associated with a lean body condition, while IL-15 deficient mice gained significantly more weight, developing an obese phenotype when compared to control C57Bl/6 (B6) mice. We furthered these observational studies to demonstrate that acute IL-15 administration resulted in weight loss in IL-15-/- mice and in a relevant murine model of diet induced obesity. Furthermore, we found that the circulating levels of IL-15 were significantly decreased in human obese individuals compared to lean subjects.

Dr. Ali Ashkar and I were responsible for the design and interpretation of the experiments. Sarah Reid, Randy MacKenzie, and I were responsible for the generation of the data. I was responsible for the analysis of the data presented. Dr. Geoff Werstuck provided the human blood samples. I and Drs. Ali Ashkar, Alison Holloway, Bernardo Trigatti, and Carl Richards contributed to the writing and editing of this manuscript.

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INTERVENTION AND PREVENTION

# Interleukin-15 Contributes to the Regulation of Murine Adipose Tissue and Human Adipocytes

Nicole G. Barra[1], Sarah Reid[1], Randy MacKenzie[1], Geoff Werstuck[2], Bernardo L. Trigatti[3,4], Carl Richards[1], Alison C. Holloway[5] and Ali A. Ashkar[1]

**An alarming global rise in the prevalence of obesity and its contribution to the development of chronic diseases is a serious health concern. Recently, obesity has been described as a chronic low-grade inflammatory condition, influenced by both adipose tissue and immune cells suggesting proinflammatory cytokines may play a role in its etiology. Here we examined the effects of interleukin-15 (IL-15) on adipose tissue and its association with obesity. Over expression of IL-15 (IL-15tg) was associated with lean body condition whereas lack of IL-15 (IL-15$^{-/-}$) results in significant increase in weight gain without altering appetite. Interestingly, there were no differences in proinflammatory cytokines such as IL-6 and tumor necrosis factor-α (TNF-α) in serum between the three strains of mice. In addition, there were significant numbers of natural killer (NK) cells in fat tissues from IL-15tg and B6 compared to IL-15$^{-/-}$ mice. IL-15 treatment results in significant weight loss in IL-15$^{-/-}$ knockout and diet-induced obese mice independent of food intake. Fat pad cross-sections show decreased pad size with over expression of IL-15 is due to adipocyte shrinkage. IL-15 induces weight loss without altering food consumption by affecting lipid deposition in adipocytes. Treatment of differentiated human adipocytes with recombinant human IL-15 protein resulted in decreased lipid deposition. In addition, obese patients had significantly lower serum IL-15 levels when compared to normal weight individuals. These results clearly suggest that IL-15 may be involved in adipose tissue regulation and linked to obesity.**

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## INTRODUCTION

Obesity continues to rise in both industrialized and developing countries, and is now a global epidemic (1). The prevalence of obesity has exceeded 20% recently in many European countries (2). In North America, it is estimated that >30% of adults are obese (3) coupled with alarming rises in obesity rates in children (4). Because obesity is a major risk factor in the development of numerous chronic diseases such as cancer, cardiovascular disease, and diabetes mellitus, obesity has and will continue to place a substantial burden on health-care systems (1,5). However, treatment of obesity is difficult as the pathophysiology related to this disease is complex and not well-understood.

Prior to the discovery of leptin and adiponectin, adipocytes were viewed primarily as energy storing cells with limited functions. However, it is becoming increasingly evident that these cells are highly active and are able to secrete various factors that have proinflammatory and anti-inflammatory properties (6–8). Increasing evidence suggests a strong link between adipose tissue and the immune system in the development of obesity. Obesity is characterized as a chronic low-grade inflammatory condition, therefore proinflammatory cytokines may play a role in its etiology (6). In support of this hypothesis it has been shown that adipocytes actively secrete inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (8) both of which are known to affect energy metabolism (6,9). However, other cytokines may also be involved in the dysregulation of energy balance that is characteristic of obesity. A recent study showed absence of IL-18 results in hyperphagia, and increased weight gain and lipid deposition (10). Another potential target is IL-15. IL-15 was first described as a cytokine which enhanced antitumor responses and stimulated T-lymphocyte proliferation (11). As a type I cytokine, its ability to activate and enhance natural killer (NK) cell cytotoxicity demonstrates its importance in innate immunity (12). Although it is best known as an immunomodulatory cytokine, IL-15 has also been shown to have effects outside the immune system in both skeletal muscle and adipose tissues, affecting lean/fat body composition (13).

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The expression of IL-15 mRNA and its receptor in skeletal muscle (14) and adipose tissue (15) suggests that this cytokine also has a role in energy metabolism. Indeed, it has been shown that IL-15 administration causes significant reductions in adipose tissue deposition in rats without altering food intake (13). Quinn *et al.* (16) also showed that IL-15 treatment inhibited lipid deposition in differentiated murine 3T3-L1 adipocytes. Finally, IL-15 has been shown to upregulate lipid-related transcription factors (peroxisome proliferator–activated receptors δ and α), thermogenic proteins (UCP1 and UCP3) as well as other proteins involved in membrane and mitochondrial transport of fatty acids in brown adipose tissue (17). To date, the effects of IL-15 on human adipocytes and murine gene expression in white adipose tissue have not been examined. As well, possible IL-15 baseline levels in human obese and control serum samples may help explain obese disease susceptibility.

In this study, we sought to determine the role of IL-15 on weight gain and adiposity in knock out, control, and transgenic (tg) mice. Second, to determine whether IL-15 can induce weight loss in two mouse models: diet-induced obese and IL-15$^{-/-}$ mice. Third, we measured lipid deposition in differentiated human adipose-derived stem cells treated with IL-15, as well as baseline IL-15 levels in obese patients to determine any association between IL-15 expression and human obesity.

## METHODS AND PROCEDURES

### Animals

Female C57BL/6 (B6) mice, 6–8 weeks old, were purchased from Charles River Laboratory (Montreal, Quebec, Canada) and placed into two groups. The high fat group received a diet containing 60 fat, 20 protein, and 20% carbohydrate (D12492, Research Diets, New Brunswick, NJ) for 30 weeks, whereas other mice were given a mouse breeder diet. A breeding pair of IL-15tg mice on a C57BL/6 background was kindly provided by M. Caligiuri (Ohio State University, School of Medicine, Columbus, OH). These mice were then bred in the barrier facilities at McMaster University. IL-15$^{-/-}$ mice were purchased from Taconic (Germantown, NY). All mice were maintained at McMaster University's Central Animal Facility. All animal experiments were approved by the Animal Research Ethics Board at McMaster University. Mice were housed in level B rooms and were maintained under controlled lighting (12:12 L:D) and temperature (22 °C) with *ad libitum* access to food and water.

### Body weight and food consumption

IL-15$^{-/-}$, IL-15tg, and control B6 mice were weighed once a week for 6 months to assess differences in body weight. Diet-induced obese B6 mice were weighed once a week for 30 weeks. Food consumption was measured twice for a period of five consecutive days. Preweighed food was placed in food hoppers and measured daily on a per-cage basis. Food intake was determined as grams consumed per gram of mouse per day. During IL-15 treatment, body weight and food consumption was monitored daily from the onset of treatment until mice were killed.

### Delivery of IL-15

Percentage of weight loss was recorded when mice were delivered IL-15 in two different ways. 500 ng of recombinant murine IL-15 (R&D Systems, Minneapolis, MN) was injected per mouse for seven consecutive days intraperitoneally in IL-15$^{-/-}$ mice. The human IL-15 gene was optimized and generated in an Ad-expressing vector, Opt.hIL-15 (E.J. Davies, S. Reid, M. Medina, B.D. Lichty, A.A. Ashkar, unpublished data). The empty adenoviral vector, Ad-d170.3, was a gift from the Robert E. Fitzhenry Vector Laboratory (Centre for Gene Therapeutics, Hamilton, ON). Six- to 8-week old female diet-induced obese B6 mice were administered with $5 \times 10^8$ plaque-forming units Opt.hIL-15 or Ad-d107.3, or 300 µl phosphate-buffered saline (PBS) via IV tail injections on day 0, 3, and 6. Mice were anaesthetized and killed on day 8 after a 16 h fast.

### Cell isolation from fat tissue for FACS analysis

Epididymal fat pads from mice were isolated, washed in PBS, minced, and digested in collagenase A (Roche Applied Science, Laval, Quebec, Canada) for 30 min at 37 °C. The cell suspension was filtered through a 100 µm, followed by a 70 µm and 40 µm cell strainer to remove tissue debris. This solution was then centrifuged for 10 min at 1,200 rpm at 4 °C, resuspended in ACK lysis buffer to remove red blood cells, and washed in PBS. Adipose-derived cells were counted using a hemocytometer and resuspended in a 0.2% bovine serum albumin PBS solution for FACS analysis. Five-hundred thousand cells per well were plated in a 96 well plate. After cells were washed and blocked using CD16/CD32 antibody (eBioscience, San Diego, CA), they were surfaced stained with fluorescein isothiocyanate– and phycoerythrin-conjugated anti-mouse CD45.2 (clone 104, eBioscience) and NK1.1 antibodies, respectively (clone PK136, BD Pharmingen, San Diego, CA). Stained cells were analyzed on a FACSCanto flow cytometer collecting 100,000 gated events. FlowJo flow cytometry analysis software was used for FACS analysis.

### Tissue collection, histology, and cell quantification

Epididymal, mesenteric, and adrenal fat sources, referred to as visceral fat, were excised from IL-15$^{-/-}$, IL-15tg, and C57Bl/6 mice and weighed. Epididymal fat pads from the three strains of mice were fixed in paraformaldehyde, embedded in paraffin, cross-sectioned, and stained with hematoxylin and eosin and measureable parameters, such as cell areas, were analyzed using AxioVision software (created by Carl Zeiss MicroImaging). Blood was collected, placed in 4 °C over night, and centrifuged at 5,000 rpm for 10 min to collect serum.

### Cell culture

Human adipose-derived stem cells isolated from human lipoaspirate tissue were purchased from Invitrogen. Cells were cultured and expanded in MesenPRO RS Medium and growth supplement provided by Invitrogen. Cells at 2–4 passages were used for experiments. Because these cells have the potential to differentiate into several phenotypes, 38,000 cells plated in a 24 well plate were induced using adipogenic differentiation media containing 10% fetal bovine serum, 2 mmol/l L-glutamine, 10 µg/ml human insulin (Invitrogen, Carlsbad, CA), 1 µmol/l dexamethasone, 100 µmol/l indomethacin, 0.5 mmol/l 3-isobutyl-1-methylxanthine (Sigma, Oakville, Ontario, Canada), and 5 µg/ml penicillin/streptomycin in high glucose Dulbecco's modified Eagle medium (Invitrogen). Cells were treated with 0, 250, or 500 ng/ml of recombinant human IL-15 (Peprotech, Rocky Hill, NJ) every 2 days for a period of 14 days. Oil Red O staining methods assessed lipid deposition after various treatments with recombinant human IL-15 as previously described (16).

### RNA isolation and reverse transcriptase-PCR

Total RNA was isolated from noninduced adipose-derived stem cells and human adipocytes differentiated and treated with either 0, 250, or 500 ng/ml of IL-15. Isolated RNA was treated for 30 min at 37 °C with RNase-free Dnase (Ambion, Foster City, CA). PCR was performed with PCR supermix (Invitrogen) containing Taq DNA polymerase. Primer sets used for PCR analyses include: peroxisome proliferator–activated receptor-γ forward GGCCGCAGATTTGAAAGAAG and reverse ATTCAATT GCCATGAGGGAG; sterol regulatory element binding protein 1c forward ACCGACATCGAAGGTGAAGT and reverse ATCTTCAA TGGAGTGGGTGC; lipoprotein lipase forward GTCCGTGGCTA CCTGTCATT and reverse TGGATCGAGGCCAGTAATTC, fatty acid synthase forward TGGTTCATCTGCTCTGGGAT and reverse CTGCT CCACGAACTCAAACA; and fatty acid binding protein (aP2) forward TGCAGCTTCCTTCTCACCTT and reverse TGGTGCTCTTGACTT TCCTG.

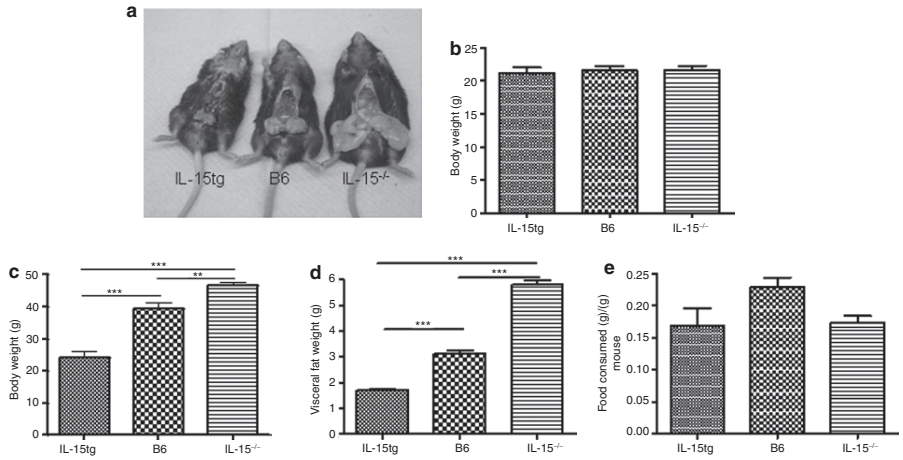


**Figure 1** Varying expression of interleukin-15 (IL-15) cause significant differences in weight gain without altering food consumption. (**a**) Picture shows roughly age matched male control B6 alongside an IL-15tg and IL-15$^{-/-}$ mouse with epididymal fat pads excised. (**b**,**c**) Bar graphs show body weights of female 6–8-week-old B6 ($n = 7$), IL-15tg ($n = 7$), and IL-15$^{-/-}$ ($n = 7$) mice in grams monitored weekly at 8 week and 6 month time points. (**d**) Visceral fat weights are also shown in grams, made up of epididymal, adrenal, and mesenteric fat sources. (**e**) Bar graph shows food consumed per gram of mouse monitored twice for a five consecutive day period in B6 ($n = 8$), IL-15tg ($n = 12$), and IL-15$^{-/-}$ ($n = 16$). $^{**}P < 0.01$, $^{***}P < 0.001$.

### ELISAs for human IL-15 and murine IL-6 and TNF-α

Serum samples were collected from clinically obese and normal weight individuals based on BMI, and from treated and control mice. Human IL-15 levels in mice and humans were quantified using hIL-15 DuoSet ELISA kit (R&D Systems) and hIL-15 Quanti-Glo ELISA kit (R&D Systems), respectively. Also, proinflammatory cytokines TNF-α and IL-6 (IL-6) were quantified in mice using DuoSet ELISA kits (R&D Systems).

### Statistics

Significant differences between two groups were determined using Student's *t*-tests. Greater than two group comparisons were made using one-way ANOVA followed by Tukey's *post hoc* multiple comparisons test. All tests were done using Graph Pad Prism 4 software.

## RESULTS

### Presence or absence of IL-15 affects body weight

Body weights of B6 mice with varying levels of IL-15 expression were monitored to determine whether IL-15 had an effect on body weight (Figure 1). A clear physical difference is shown in both the size of epididymal fat pads and mouse between a control B6 and IL-15$^{-/-}$ compared to the IL-15tg (Figure 1a). Although no differences were seen after 8 weeks (Figure 1b), significant differences in body weights were seen at 6 months with over expression of IL-15 resulting in significantly lower body weight compared to control B6 mice whereas IL-15$^{-/-}$ mice experienced significant weight gain when compared to controls (Figure 1c). Significant differences in visceral fat weight, which include the addition of epididymal, mesenteric, and adrenal fat sources, were observed showing IL-15tg mice had the least weight followed by B6 and IL-15$^{-/-}$ (Figure 1d). Because we observed significant differences in body weight,

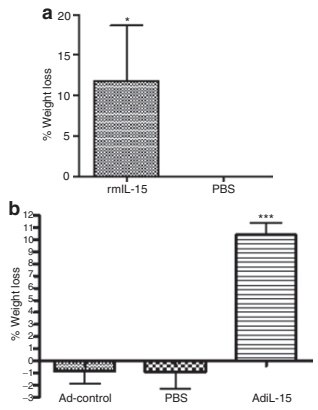


**Figure 2** Interleukin-15 (IL-15) treatment induces weight loss in IL-15$^{-/-}$ and in diet-induced obese mice. (**a**) Bar graph shows percentage of weight loss in IL-15$^{-/-}$ mice treated with either 500 ng of rmIL-15 or 300 µl of PBS for 7 consecutive days ($n = 5$ per group). (**b**) Percentage of weight loss in diet-induced obese B6 mice treated with either phosphate-buffered saline (PBS), Ad-control, and AdIL-15 ($n = 5$ per group). $^{*}P < 0.05$, $^{***}P < 0.001$.

food consumption rates were determined between IL-15tg, IL-15$^{-/-}$, and B6 controls in order to determine whether varying levels of IL-15 affected central regulation of appetite. There were no significant differences in food consumption

between the three strains (Figure 1e). Finally, because IL-15 is a proinflammatory cytokine, we wanted to determine whether the presence or absence of IL-15 in our three strains of mice correlated to increased systemic expression of other inflammatory cytokines such as TNF-α and IL-6. We were unable to detect serum IL-6 or TNF-α in our three strains of mice (data not shown).

### IL-15 treatment leads to weight loss

Because we observed that absence of IL-15 leads to higher fat deposition and overexpression of IL-15 leads to a lean body condition, we then examined whether delivery of recombinant murine IL-15 affects body weights *in vivo*. Treatment with rmIL-15 resulted in significant weight loss in IL-15$^{-/-}$ comparable to PBS treated IL-15$^{-/-}$ mice (Figure 2a). To determine whether weight loss could be induced in an obese mouse model, B6 mice placed on a high fat diet were treated with an adenoviral construct optimizing IL-15 secretion, referred to as "AdIL-15," which expressed 4849.3 pg/ml (±1190.72 pg/ml s.d.) of human IL-15 in circulation. At 30 weeks, these mice weighed ~54.21 g (±2.0545 g s.d.). AdIL-15 treatment resulted in significant weight loss when compared to mice injected with either an empty adenoviral construct referred to as "Ad-Control" or PBS (Figure 2b). This suggests that the effects of IL-15 on adiposity are directly mediated on possibly adipocytes themselves.

### Over expression of IL-15 leads to less fat deposition and increased NK1.1$^+$ cell accumulation in adipose tissue

Because we observed that IL-15 has significant effects of fat mass, we then examined cross-sections of abdominal fat pads in order to determine whether the decrease in both the size and mass of these fat pads due to IL-15 treatment may be attributed to either lower cell numbers or decreased cell size and fat content. These sections demonstrate that over expression of IL-15 cause adipocytes to become very small, resulting in decreased cell areas compared to B6 and IL-15$^{-/-}$ mice (Figure 3).

Quinn *et al.* (16) demonstrated 3T3-L1 cells do not express IL-15 at any stage of differentiation, suggesting that IL-15 may mediate its affects directly on adipocytes. Because it is known that IL-15 exerts its immunological fuctions via NK cells and delivery of IL-15 to IL-15$^{-/-}$ mice also reconstitutes NK cells, we wanted to determine whether over expression of IL-15 resulted in increased accumulation of NK cells in adipose tissue. Using collagenase digestion, we determined the percentage of NK1.1$^+$ stained cells from epididymal fat pads of our three strains of mice. These cells were gated off a leukocyte CD45.2$^+$ population. Figure 3e shows representative histograms from our three murine strains. Although the percentage of CD45.2$^+$ cells in adipose are similar, over expression of IL-15 resulted in an increase in the percentage of NK1.1$^+$ cells in epididymal fat pads followed by B6 and IL-15$^{-/-}$ (Figure 3f). This suggests

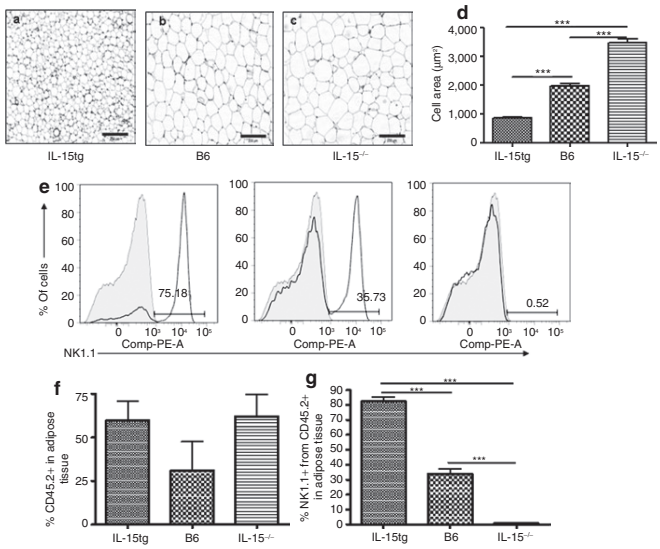


**Figure 3** Varying interleukin-15 (IL-15) expression affects adipocyte size and natural killer (NK) cell accumulation in adipose tissue. Representative cross-sections of epididymal fat pads excised from (**a**) IL-15tg, (**b**) B6, and (**c**) IL-15$^{-/-}$ mice were stained with hematoxylin and eosin. (**d**) Bar graph shows cell areas analyzed using the AxioVision software ($n = 6$ per group). (**e**) Analysis of NK1.1$^+$ cells gated from a CD45.2$^+$ population in IL-15tg, B6, and IL-15$^{-/-}$ mice. The percentage of (**f**) CD45.2$^+$ cells and (**g**) NK1.1$^+$CD45.2$^+$ from the analyzed population are shown. (IL-15tg $n = 3$, B6 $n = 4$, IL-15$^{-/-}$ $n = 4$). ***$P < 0.001$.

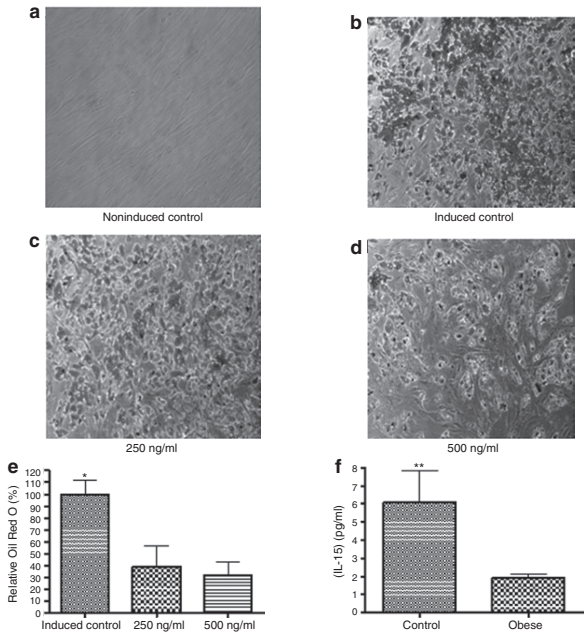


**Figure 4** Baseline interleukin-15 (IL-15) serum levels lower in obese individuals compared to controls, and IL-15 treatment results in decreased lipid deposition in human adipose-derived stem cells, demonstrating an association between IL-15 and obesity. (**a**) Cells not treated with adipogenic media were stained with Oil Red O. Human preadipocytes were treated every 2 days with either (**b**) 0, (**c**) 250, or (**d**) 500 ng/ml of recombinant human IL-15 at time of induction (day 0) and were stained with Oil Red O. Pictures were taken under a ×10 objective. (**e**) Relative differences in lipid deposition were confirmed using isopropanol extracted Oil Red O stain at day 14 ($n = 3$ per group). (**f**) Serum collected from average weight control individuals ($n = 24$) and obese patients ($n = 40$) were analyzed for IL-15 expression using a hIL-15 Quanti-Glo ELISA kit. $^*P < 0.05$, $^{**}P < 0.01$.

that IL-15 may potentially mediate some of its antiadipogenic effects through an immune mediated mechanism via NK cells.

### IL-15 inhibit lipid deposition in human primary adipocytes

To test whether human IL-15 can directly influence lipid deposition in human adipocytes, we stimulated human adipose-derived stem cells to differentiate into adipocytes in the absence or the presence of different concentrations of IL-15. We stained neutral lipid droplets with Oil Red O, extracted the stain with isopropanol and measured the concentration of extracted stain spectrophotometrically as a measure of the neutral lipid content of cells (Figure 4a–d). IL-15 caused decreased lipid deposition upon differentiation of human adipose-derived stem cells (Figure 4e). Similar results occurred when cells were treated 2 days after induction; however, treatment on and after day 4 resulted in no differences between groups (data not shown). Differences in expression of adipogenic transcription factors such as peroxisome proliferator–activated receptor-γ and sterol regulatory element binding protein 1c, as well as fat specific genes such as lipoprotein lipase, fatty acid synthase, and fatty acid binding protein (aP2) due to IL-15 treatment could not be determined using reverse transcriptase-PCR (data not shown).

**Table 1 Characteristics of human subjects used in measuring baseline serum interleukin-15 levels**

| Measurement | Lean | Obese |
|---|---|---|
| Sex ratio (male:female) | 11:13 | 12:28 |
| BMI (kg/m$^2$) | 23.9 (2.5) | 34.1 (5.0)*** |
| Fasting glucose (mmol/l) | 5.2 (0.7) | 5.4 (0.6) |
| Triglycerides (mmol/l) | 0.9 (0.7) | 2.0 (1.2)** |

All values represent the mean (± s.d) of lean and obese individuals.
**Difference between lean and obese individuals based on serum triglycerides ($P < 0.01$). ***Difference between lean and obese individuals based on BMI ($P < 0.001$).

### Baseline IL-15 levels in obese patients are significantly lower than controls

Obesity is a condition characterized by low-grade inflammation and elevated levels of TNF-α, a cytokine involved in systemic inflammation (18). Because TNF-α elevation is correlated with increased adiposity, we sought to determine whether there is an association between IL-15 levels and obesity in human

subjects. Clinical and biochemical characteristics from each group are shown in **Table 1**. **Figure 4f** showed circulating IL-15 baseline levels were significantly lower in obese individuals compared to controls.

## DISCUSSION

In this study, we investigated the effect of IL-15 on adipose both *in vivo* and *in vitro*, providing several lines of evidence supporting a role for IL-15 as a potent regulator of adipose tissue. First, over expression of IL-15 significantly decreases the ability for tg mice to gain weight, whereas lack of this cytokine results in significant increase in weight gain without altering appetite. Second, treatment with rmIL-15 and AdIL-15 results in significant weight loss in IL-15$^{-/-}$ and diet-induced obese control B6 mice, respectively. More importantly, the effect of IL-15 on body weight was independent of food intake, which has been previously shown (13). Third, cross-sections of murine fat pads show significant decrease in fat deposition in adipocytes which results to adipocyte shrinkage with over expression of IL-15. Finally, treatment of human adipocytes with hrIL-15 significantly inhibited fat deposition. Furthermore, obese patients have decreased serum IL-15 levels when compared to normal weight individuals, linking IL-15 with human obesity.

Although IL-15 is a potent inducer of NK cell activation (12), IL-15 treatment reduces lipid accumulation in human adult stem cell derived adipocytes and in murine 3T3-L1 adipocytes in culture (16), suggesting that the effects of IL-15 on adiposity may be a consequence of a direct effect of IL-15 on adipocytes. These results are in agreement with Carbó *et al.* (13) hypothesis that this cytokine may act as a potential regulator of fat mass through its secretion by skeletal muscle, thus affecting lean/fat body composition. The actual amount of IL-15 secreted by skeletal muscle in normal and obese individuals and the stimuli involved in its secretion would help strengthen this hypothesis. On the other hand, although skeletal muscle contains the greatest amount of tissue IL-15 mRNA, other tissues as the heart and kidney, and immune cells including monocytes and macrophages also express IL-15 mRNA, suggesting that other tissues may also be involved (11). For example, the recent demonstration that macrophages are present in adipose tissue and influence adipocyte function (19) suggests that they may be an important source of IL-15 secreted locally within adipose tissue. In addition to a direct effect on adipocytes themselves, IL-15 treatment in mice has also been shown to result in reduced lipoprotein lipase activity as well as alter intestinal lipid absorption (15,20). These effects may also contribute to weight loss in mice with IL-15 overexpression or treated mice and the increased obesity in IL-15$^{-/-}$ mice.

Similar to our differentiated human adipocyte results from mesenchymal stem cells, Quinn *et al.* showed IL-15 treatment of murine adipogenic 3T3-L1 had no effect on lipid deposition when given 4 days after induction (16). These *in vitro* results, as well as our cross-sections of IL-15tg epididymal fat, suggest IL-15 can only affect lipid deposition in early stages of adipocyte differentiation. It remains to be clarified how IL-15 alters genetic expression of lipogenic markers in differentiated human adipocytes, resulting in decreased lipid deposition. However, we (data not shown) and others (13,15) have shown that IL-15 can exert affects on differentiated adipocytes. Although conflicting, these results together not only suggest IL-15 may affect adipocytes at different stages of differentiation, but may affect adipocyte turnover rate. It has been shown in human subjects ~10% of adipocytes are renewed annually and the number of adipocytes is predetermined at adolescence and remains stable throughout adulthood (21).

Interestingly, Caspar-Bauguil *et al.* (22) characterized the distribution of lymphocytes in both epididymal and subcutaneous inguinal adipose sites in control B6 mice. Similar to our results in control B6 mice, this study demonstrates that a high percentage of NK cells are found within the lymphocyte population in epididymal fat. As seen in our IL-15tg mice, the overexpression of IL-15 resulted in an accumulation of NK cells in epididymal fat. This suggests that not only does the expression of IL-15 affect NK cell accumulation in epididymal fat sources, but IL-15 may also affect other subpopulations of lymphocytes located in this and other fat sources.

Associations between obesity and other inflammatory markers, such as TNF-α, have been previously made (9,18). Netea *et al.* (10) showed significant weight gain resulting from IL-18 deficiency caused increased food intake, demonstrating similar effects in energy intake as leptin. Similar to our results, lack of IL-15 caused significant weight gain. We have found lower levels of IL-15 in serum from obese individuals when compared to controls. Similar findings have been reported by Nielsen *et al.* (23). These findings clearly link IL-15 to human obesity. Obese individuals are more likely to develop cancer and other chronic diseases compared to normal weight counterparts (1,5,24). It has been shown that obese patients have lower number of NK cells when compared to non-obese groups (25). Because IL-15 is critical in the development and function of NK cells (26), lower serum IL-15 in obese patients is likely associated with fewer frequencies and lower activities of NK cells, possibly resulting in the increased susceptibility to tumors and development of some chronic diseases associated with obesity. Similar to our IL-15$^{-/-}$ mice, Caspar-Bauguil *et al.* (22) have shown that the percentage of NK cells located in epididymal fat pads decreases in mice placed on a high fat diet. Because obesity is described as a chronic low-grade inflammatory state, the ability of IL-15 to alter this state as well as possibly prevent the development of chronic diseases still needs to be examined.

In conclusion, over expression of IL-15 results in lean body condition, whereas lack of IL-15 results in significant increase in weight gain. Treatment with IL-15 induces weight loss without altering food consumption by affecting lipid deposition in adipocytes. Additionally, serum IL-15 is significantly lower in obese patients when compared to normal weight individuals, demonstrating an association with body fat mass. Therefore, these results suggest that IL-15 may be involved in adipose tissue regulation and obesity.

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**DISCLOSURE**

The authors declared no conflict of interest.



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**CHAPTER 3**

## Interleukin-15 Treatment Induces Weight Loss Independent of Lymphocytes

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This manuscript examines whether IL-15 mediated weight loss occurs indirectly through immune lymphocytes. We were able to determine that IL-15 administration altered the immune adipose tissue environment while inducing weight loss in B6 mice. Through the use of RAG2-/-$\gamma_c$-/- mice and NK-depleting antibodies we were able to assess that IL-15 mediated weight loss occurs independently of lymphocytes and of signalling through the common $\gamma_c$.

Dr. Ali Ashkar and I were responsible for the design and interpretation of the experiments. Marianne Chew, Sarah Reid, and I performed the experiments, while I analyzed the data and wrote the paper. All authors contributed to the editing of this manuscript.

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# Interleukin-15 Treatment Induces Weight Loss Independent of Lymphocytes

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## Abstract

Obesity is a chronic inflammatory condition characterized by activation and infiltration of proinflammatory immune cells and a dysregulated production of proinflammatory cytokines. While known as a key regulator of immune natural killer (NK) cell function and development, we have recently demonstrated that reduced expression of the cytokine Interleukin-15 (IL-15) is closely linked with increased body weight and adiposity in mice and humans. Previously, we and others have shown that obese individuals have lower circulating levels of IL-15 and NK cells. Lean IL-15 overexpressing (IL-15 tg) mice had an accumulation in adipose NK cells compared to wildtype and NK cell deficient obese IL-15$^{-/-}$ mice. Since IL-15 induces weight loss in IL-15$^{-/-}$ and diet induced obese mice and has effects on various lymphocytes, the aim of this paper was to determine if lymphocytes, particularly NK cells, play a role in IL-15 mediated weight loss. Acute IL-15 treatment resulted in an increased accumulation of NK, NKT, and CD3$^+$ T cells in adipose tissue of B6 mice. Mice depleted of NK and NKT cells had similar weight loss comparable to controls treated with IL-15. Finally, IL-15 treatment induces significant weight loss in lymphocyte deficient RAG2$^{-/-}\gamma c^{-/-}$ mice independent of food intake. Fat pad cross-sections show decreased pad size with cytokine treatment is due to adipocyte shrinkage. These results clearly suggest that IL-15 mediates weight loss independent of lymphocytes.

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## Introduction

Obesity is defined as an accumulation of adipose tissue and is associated with systemic chronic inflammation [1]. Altered immune responses in obese individuals have been recently linked to the development of comorbidities such as insulin resistance and dyslipidemia [1–3]. As an inflammatory condition, obesity is characterized by increased infiltration of various immune cells into adipose tissue, such as M1 polarized macrophages, as well as a decrease in anti-inflammatory immune cells such as M2 polarized macrophages [2,4]. As prevalence rates and its associated chronic conditions continue to rise, insights into the pathophysiology related to obesity and the immune contribution to metabolic disease development is essential in formulating novel therapeutic strategies in treating obesity and its associated comorbidities.

Although several reports have examined the role of altered adipose macrophage phenotypes in obese individuals [2,4,5], recent studies have also demonstrated associations between obesity and alterations in lymphocyte populations. In obese adipose tissue, increased CD8$^+$ T cells and decreased CD4$^+$ and anti-inflammatory regulatory T cells have been found [6,7]. Obese adipose tissue has been shown to activate CD8$^+$ T cells leading to macrophage recruitment and activation [7]. As well, natural killer T (NKT) cells have been shown to play a role in metabolic abnormalities associated with obesity [8,9]. Lastly, innate immune natural killer (NK) cells have reduced cytotoxicity in obese animals compared to lean controls [10]. Alterations in these lymphocyte populations in obese individuals suggest the presence or absence of these cell types may play a role in weight regulation. Since a dysregulation in the production of proinflammatory cytokines also contributes to this inflammatory state through such factors as interleukin-6 (IL-6) and tumor necrosis factor –α (TNF-α) [11], determining the role of these cytokines in regulating immune lymphocyte responses in obesity is also essential in formulating treatments for obese individuals.

While best known as a key regulator of immune natural killer cell function and development [12,13], we and others have recently demonstrated that reduced expression of the cytokine Interleukin-15 (IL-15) is closely linked with increased body weight and adiposity in both mice and humans [14–19]. Previous work has shown that IL-15 treatment causes a significant decrease in adipose tissue mass in normal weight [19,20] and obese rodents [18], reduces lipoprotein lipase expression [18], and may play a role in lipid oxidation [21,22]. We have previously observed that over expression of IL-15 (IL-15 tg) in mice was associated with a lean body condition, while mice lacking IL-15 (IL-15$^{-/-}$) gained significantly more weight, developing an obese phenotype when compared to control C57BL/6 (B6) mice. Also, we found that IL-15 tg mice had increased percentage of NK cells found in adipose tissue compared to normal weight B6 mice and their heavier NK cell deficient IL-15$^{-/-}$ counterparts [14]. As well, obese individuals and mice placed on high fat diets have decreased

NK cell numbers in circulation and in adipose tissue [14,23]. We have also demonstrated that IL-15 treatment induces weight loss in diet-induced obese mice and in IL-15$^{-/-}$ mice, which has also been shown to reconstitute NK cell populations [14,24]. As well, IL-15 is known to activate other lymphocytes such as NKT cells [25,26] and T cells [26,27]. Whether NK cells and/or other lymphocytes contribute to the weight loss effects exerted by IL-15 treatment has yet to be determined.

In this study, we sought to determine if NK cells mediate weight loss in IL-15 treated animals. We first determined if NK cells, along with other lymphocytes such as NKT and T cells, accumulate in adipose tissue with acute IL-15 treatment in control B6 mice. In order to determine if IL-15 induces weight loss indirectly via NK cell activation, we depleted mice of NK cells using a NK1.1 cell depleting antibody, treated with IL-15, and monitored for weight loss. In order to determine the importance of lymphocyte activation via IL-15 treatment, we also employed the use of the RAG2$^{-/-}\gamma c^{-/-}$ mouse model, which has no lymphocytes and lacks the γ-receptor subunit. Altogether, these experiments will determine the role of lymphocytes, specifically NK cells, in IL-15 regulation of adipose tissue.

## Methods

### Ethics

All animal experiments were approved by the Animal Research Ethic Board (AREB) of McMaster University. The AREB approval number is: 10-02-12.

### Animals

Sixteen week old C57BL/6 (B6) female mice were purchased from Charles River Laboratory (Quebec, Canada). Female lymphocyte deficient Balb/c RAG-2$^{-/-}\gamma c^{-/-}$ mice were bred from breeding pairs given as a gift by M. Ito (Central Institute for Experimental Animals, Kawasaki, Japan) and maintained at McMaster University's Central Animal Facility. Null mutation of the RAG2 gene prevents B and T lymphocyte development in these mice, while absence of the γ-chain subunit prevents NK cell maturation. Mice were caged in groups of five and maintained under controlled lighting (12:12 L:D) and temperature (22°C) with *ad libitum* access to a low fat irradiated chow diet containing 18.6% protein, 6.2% fat, and 3.5% fiber (2918, Tekland Global Diets, Indianapolis, IN) and water.

### Delivery and Detection of IL-15 and NK1.1$^{+}$ Cell Depletion Experiment

Body weights and food consumption were monitored daily from the onset of treatment until mice were sacrificed. Food consumption was measured for a period of five consecutive days. Preweighed food was placed in food hoppers and measured daily on a per-cage basis. Food intake was recorded as grams consumed per gram of mouse per day. Percentage of weight loss was recorded when mice were delivered either an Ad-expressing human IL-15 vector, Opt.hIL-15 ("AdIL-15"), or an empty adenoviral vector ("AdControl") as previously described [28]. Briefly, an 18-aa optimized signal peptide was inserted upstream of a mature hIL-15 gene by multi-step polymerase chain reactions (PCRs) using primers provided by the Molecular Biology Institute (McMaster University). After the 402-bp PCR product was isolated (Opt.hIL-15), cloned into expression vectors using *KpmI* and *XhoI* sites, Opt-hIL-15 was inserted into the adenoviral shuttle vector pDC316 and subsequently generated Ad-Op-hIL-15 pDC316 in the Robert E. Fitzhenry Vector Laboratory (McMaster Immunology Research Centre, McMaster University) [28].

B6 mice and RAG-2$^{-/-}\gamma c^{-/-}$ mice were administered with $5\times10^{8}$ pfu AdIL-15, AdControl, or 200 µl PBS via IV tail injections on day 0, 2, and 4 (n = 5 per group). For the NK1.1$^{+}$ cell depletion experiment, B6 mice were injected intraperitoneally with 200 µg of anti-mouse NK1.1 antibody (PK136 mouse immunoglobulin G2a hybridoma HB191; ATCC) daily for two days prior to AdIL-15 treatment and subsequently every three days post treatment (n = 7 per group). Mice were anaesthetized and sacrificed on day 8. Prior to sacrifice, blood was collected through the abdominal aorta and centrifuged at 5,000 rpm for 10 minutes to collect serum. Human IL-15 levels were quantified from serum using hIL-15 DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA).

### Adipose Tissue Collection, Histology, and Cell Quantification

Visceral gonadal fat pads from RAG-2$^{-/-}\gamma c^{-/-}$ mice were weighed and fixed in paraformaldehyde. Tissues were then embedded in paraffin, and two cross-sections per mouse were stained with hematoxylin and eosin (H&E). For each cross section, 8 fields of view were quantified for cell areas using AxioVision software (created by Carl Zeiss MicroImaging).

### Cell Isolation from Fat Tissue for FACS Analysis

Weighed visceral gonadal fat pads from B6 mice were isolated, washed in PBS, minced, and digested in collagenase A (Roche Applied Science, Laval, Quebec, Canada) and 0.025 mg/ml DNase (Roche Applied Science, Laval, Quebec, Canada) for 30 min at 37°C. The cell suspension was filtered through a 100 µm, followed by a 70 µm and 40 µm cell strainer to remove tissue particulate. This solution was then centrifuged for 10 min at 1,200 rpm at 4°C. The supernatant, which included any remaining adipocytes, was discarded and the stromal vascular fraction was resuspended in ACK lysis buffer to remove red blood cells, and washed in PBS. Cells were counted using a hemocytometer and resuspended in a 0.2% bovine serum albumin PBS solution for FACS analysis. Five-hundred thousand cells per well were plated in a 96 well plate. After cells were washed and blocked using CD16/CD32 antibody (eBioscience, San Diego, CA), they were surfaced stained with fluorescein isothiocyanate–, alexa fluor 700-, and phycoerythrin-conjugated anti-mouse CD45.2 (clone 104, eBioscience), CD3 (clone 17A2, eBioscience), and NK1.1 (clone PK136, BD Pharmingen) antibodies, respectively. Stained cells were analyzed on a LSRII flow cytometer collecting 50,000 gated events and FlowJo flow cytometry analysis software.

### Statistics

All statistical analyses were performed using Graph Pad Prism 4. The results are expressed as mean ± SEM. Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* multiple comparisons test for 3 group comparisons, while Student *t*-test was used for 2 group comparisons. Significance is indicated when $p<0.05$.

## Results

### Acute IL-15 Treatment Results in an Accumulation of NK, NKT, and T Cells in Adipose Tissue

Since IL-15 is inextricably linked to NK cell function, we wanted to determine if an association between IL-15 mediated weight loss and an accumulation of NK cells in visceral gonadal fat occur in B6 mice given an acute dose of IL-15. Sixteen week old B6 mice had similar body weights between groups prior to

**Table 1.** Characteristics of mice before and after IL-15 treatment.

| | Treatment Groups | | |
|---|---|---|---|
| **Measure** | **PBS** | **AdControl** | **AdIL-15** |
| Before Treatment (B6 Mice) | 25.80±1.55 | 26.34±0.78 | 25.32±0.82 |
| After Treatment (B6 Mice) | 25.82±1.55 | 26.40±0.78 | 22.35±0.84*** |
| Before Treatment ($Rag2^{-/-}\gamma_c^{-/-}$ Mice) | 40.62±2.76 | 37.72±1.47 | 39.36±3.61 |
| After Treatment ($Rag2^{-/-}\gamma_c^{-/-}$ Mice) | 41.31±2.71 | 37.77±1.58 | 30.91±2.76** |

Data are presented as mean ± SEM. Body weights expressed as grams. Asterisks used to denote significance using a paired Student t-test comparing before and after body weights within the same group.
**P<0.01,
***P<0.001.
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treatments (Table 1). Mice treated with AdIL-15 lost significantly more weight, had sustained serum hIL-15 expression, and had higher cell numbers in the stromal vascular fraction of digested visceral fat on a per gram basis compared to mice given either AdControl or PBS (Figure 1A–C). Flow cytometric analysis revealed that IL-15 treated animals had an increase in the percentage of leukocytes ($CD45^+$), T ($CD3^+$), NK ($CD3^-NK1.1^+$) and NKT ($CD3^+NK1.1^+$) cells in visceral adipose tissue compared to controls (Figure 2). Similarly, peripheral leukocytes, T, NK, and NKT cells were also significantly elevated in spleens of IL-15 treated mice compared to controls (data not shown).

## IL-15 Treatment Induces Weight Loss Independent of NK and NKT Cells

Since IL-15 treatment results in an accumulation of NK cells in adipose tissue in B6 mice (Figure 2), we wanted to verify whether weight loss was a direct effect of IL-15 or mediated indirectly by activated NK cells. In figure 3, we show that the $NK1.1^+$ cell depleting antibody is effective in depleting NK and NKT cells from adipose tissue (Figure 3A, B). We found no significant difference in the percentage of weight loss in B6 mice treated with AdIL-15 compared to those depleted of NK and NKT cells (Figure 3C, Table 2).

## Interleukin-15 Treatment Induces Weight Loss in the Absence of Lymphocytes

Since acute IL-15 treatment resulted in an accumulation of other lymphocytic cells such as T cells, we utilized lymphocyte deficient $RAG2^{-/-}\gamma_c^{-/-}$ mice. These mice had similar body weights between groups prior to treatment (Table 1). Mice treated with AdIL-15 lost significantly more weight compared to AdControl or PBS treated animals (Figure 4A). Treatment of these mice with AdIL-15 clearly showed a marked difference in the size and weight of the abdominal visceral gonadal fat pad compared to naïve and vehicle controls (Figure 4B, C). These differences were not attributed to altered food consumption (Figure 4D).

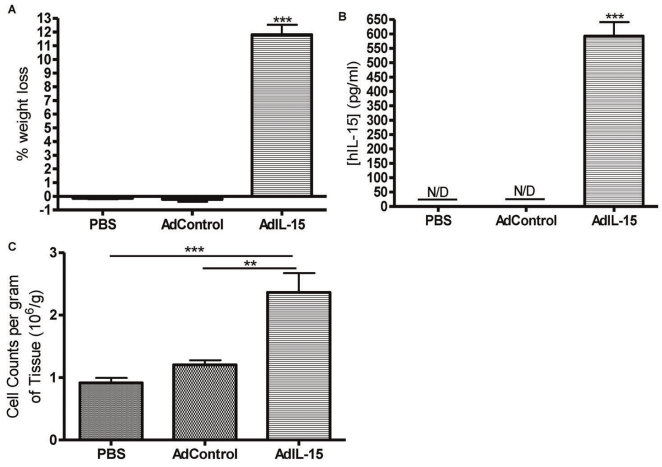


**Figure 1. Acute interleukin-15 administration results in weight loss and increased cell number in visceral adipose tissue of B6 mice.** (**A**) Bar graph shows percentage of weight loss in female B6 mice treated i.v. with either phosphate-buffered saline (PBS), an empty adenoviral construct not expressing IL-15 (Ad-Control), or IL-15 expressing adenoviral construct (AdIL-15). (**B**) Serum collected on day 8 was analyzed for IL-15 expression using a hIL-15 DuoSet ELISA kit. (**C**) The cells referred to are the number of cells in the stromal vascular fraction from digested adipose tissue in each group. The y-axis refers to the number of cells ($\times 10^6$) counted from the stromal vascular fraction per gram of digested visceral gonadal adipose tissue shown in bar graph (n = 5 per group). **P<0.01, ***P<0.001.
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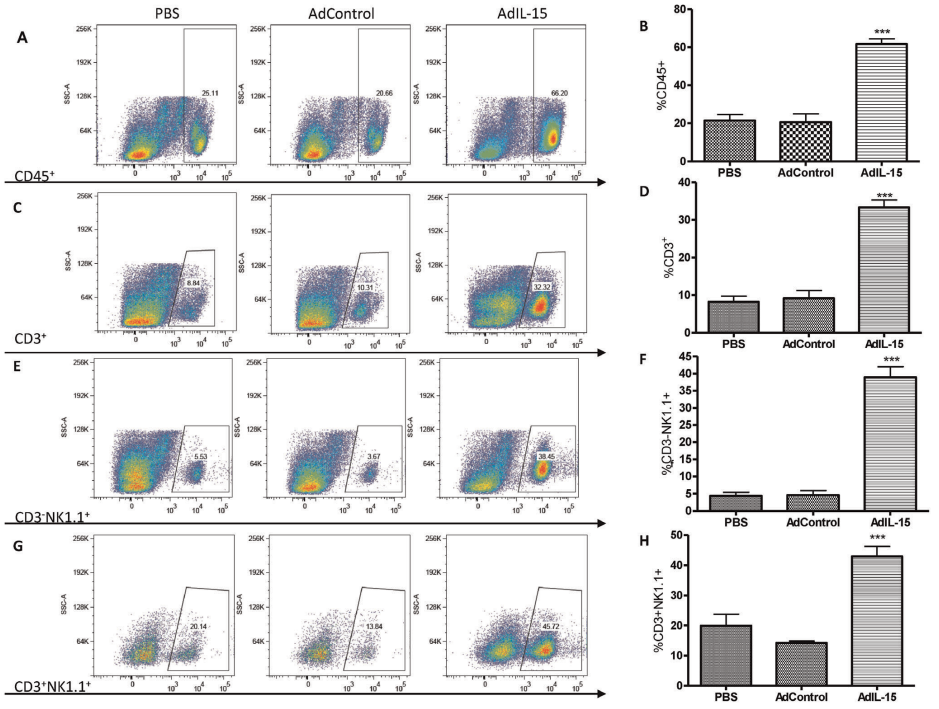


**Figure 2. Acute interleukin-15 treatment results in CD45$^+$, NK, NKT, and CD3$^+$ T cell accumulation in visceral adipose tissue in B6 mice.** Analysis of (**A**) CD45$^+$, (**C**) CD3$^+$ (T), (**E**) CD3$^-$NK1.1$^+$ (NK), and (**G**) CD3$^+$NK1.1$^+$ (NKT) cell populations in visceral gonadal adipose tissue from PBS, AdControl, and AdIL-15 treated animals. The percentage of (**B**) CD45$^+$, (**D**) CD3$^+$ (T), (**F**) CD3$^-$NK1.1$^+$ (NK), and (**H**) CD3$^+$NK1.1$^+$ (NKT) cell populations are represented as a bar graphs (n = 5 per group). ***P<0.001.
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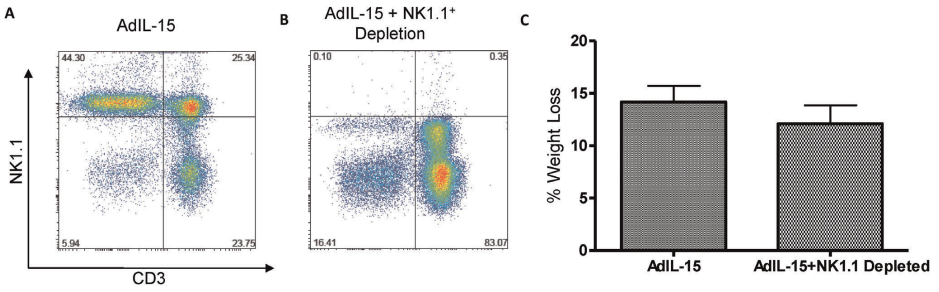


**Figure 3. Treatment with interleukin-15 induces weight loss in NK1.1$^+$ cell depleted B6 mice.** Analysis of CD3 and NK1.1 cells gated from a CD45.2$^+$ population in (**A**) AdIL-15 treated B6 mice and (**B**) NK1.1$^+$ depleted AdIL-15 treated mice in visceral gonadal adipose tissue. (**C**) Bar graph shows weight loss in female treated mice (n = 7 per group).
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**Table 2.** Characteristics of mice before and after IL-15 treatment.

| | Treatment Groups | |
|---|---|---|
| **Measure** | **AdIL-15** | **AdIL-15+NK1.1 Depletion** |
| Before Treatment (B6 Mice) | 29.18±2.65 | 29.34±1.84 |
| After Treatment (B6 Mice) | 25.09±2.49** | 25.91±1.93*** |

Data are presented as mean ± SEM. Body weights expressed as grams. Asterisks used to denote significance using a paired Student t-test comparing before and after body weights within the same group.
**P<0.01,
***P<0.001.
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### Acute IL-15 Administration Leads to Less Fat Deposition

Since we observed that IL-15 has significant effects on fat mass (Figure 4), we then examined cross sections of abdominal fat pads to assess if IL-15 treatment may be attributed to decreased cell size and fat content. These sections reveal shrunken adipocytes resulting in a lower cell area in AdIL-15-treated RAG2$^{-/-}\gamma_c^{-/-}$ mice compared to both the Ad-Control and PBS groups (Figure 5). This suggests that the effects of IL-15 on adiposity are not mediated by lymphocytes and occur independently of signaling through the common-γ-chain.

## Discussion

Results from this paper show that acute IL-15 administration results in weight loss and an accumulation of NK, NKT, and CD3$^+$ T cells in visceral adipose tissue in B6 mice. Previously, we have shown that IL-15 tg mice, which remain lean over time, have a significant increase in the percentage of NK cells found in adipose tissue compared to control B6 mice and IL-15$^{-/-}$ mice [14]. Since an accumulation of NK cells is associated with lean body weight and the effects of IL-15 are inextricably linked with NK cell function, we utilized a NK1.1 depleting antibody to determine whether IL-15 mediates weight loss directly or indirectly through NK and/or NKT cells in B6 mice. To determine if IL-15 mediates weight loss through other lymphocyte populations such as T cells, we utilized the lymphocyte deficient RAG2$^{-/-}\gamma c^{-/-}$ mouse model. IL-15 treatment resulted in weight loss, decreased visceral fat weight, and shrunken adipocytes in RAG2$^{-/-}\gamma c^{-/-}$ mice suggesting that this cytokine may directly affect adipocytes by signaling through other receptor subunits excluding the common-γ-chain.

Immune cell populations and their contribution to metabolic disease development in obesity have garnered a great deal of interest; however, whether immune cells play a role in weight regulation and adiposity has not been thoroughly examined. Lymphoid cells are in close proximity to adipocytes in various locations such as bone marrow, perinodal adipose tissue surrounding lymph nodes, and subcutaneous and visceral adipose sources [29,30]. This close contact provides opportunity for cross talk between these two cell types. Secretion of various adipokines

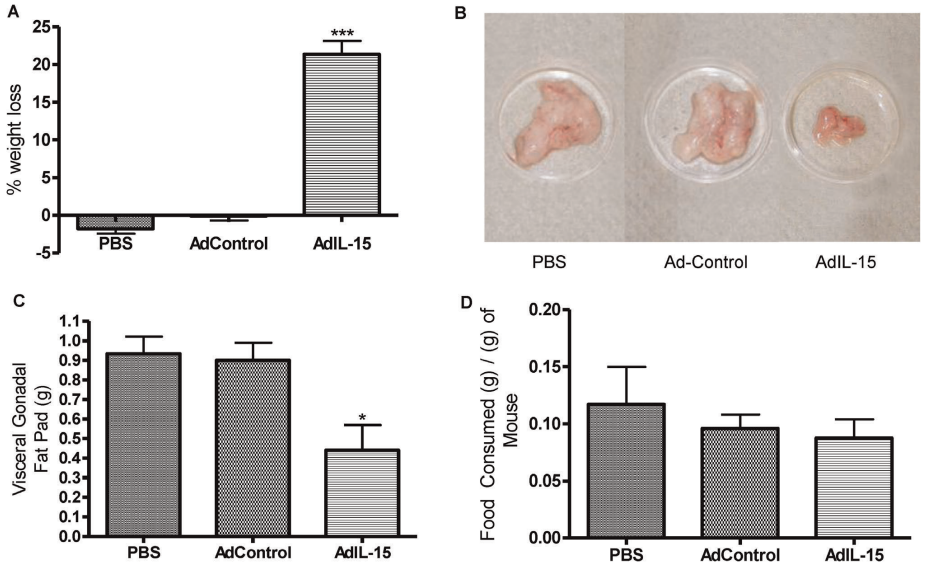


**Figure 4. IL-15 induces weight loss independent of lymphocytes and in the absence of the common gamma chain (γc).** (**A**) Percentage of weight loss represented as a bar graph. (**B**) Picture shows excised visceral gonadal fat taken from representative female naïve, Ad-Control, and AdIL-15 treated RAG2$^{-/-}\gamma c^{-/-}$ mice. (**C**) Visceral gonadal fat pad in grams shown as a bar graph (n = 5 per group). (**D**) Food consumed per gram of mouse monitored for 5 consecutive days (n = 5 per group). *P<0.05, ***P<0.001.
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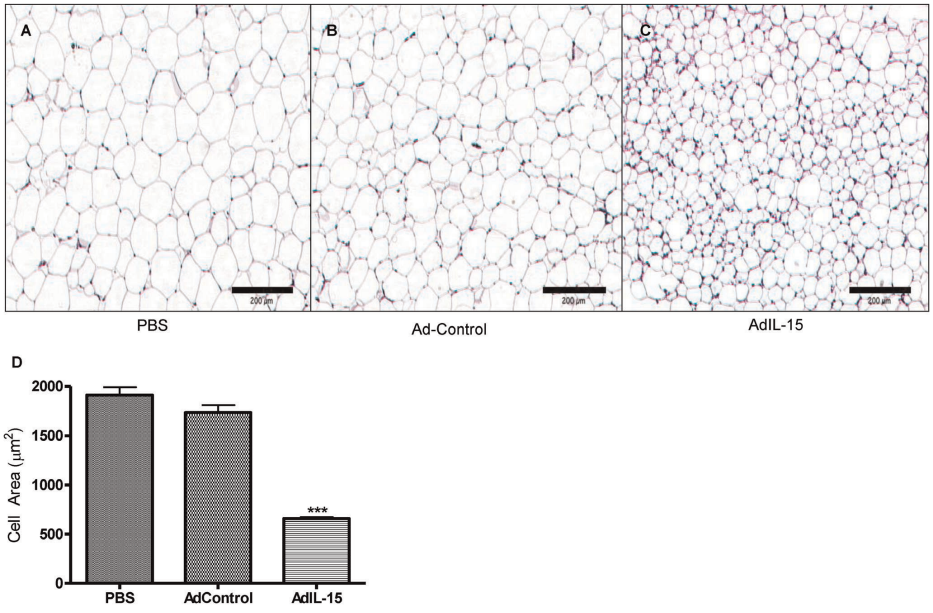


**Figure 5. IL-15 treatment affects adipocyte size in RAG2$^{-/-}\gamma c^{-/-}$ mice.** Representative cross sections of visceral gonadal fat pads excised from RAG2$^{-/-}\gamma c^{-/-}$ mice treated i.v. with (**A**) PBS, (**B**) AdControl, or (**C**) AdIL-15 were stained with hematoxylin and eosin (H&E). (**D**) Bar graph shows cell areas determined under 5× objective from each group determined using AxioVision software (n = 5 per group). ***P<0.001.
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from adipose demonstrate that this tissue may directly influence lymphocyte activity [6,7,31–33]. We were interested in determining whether factors that regulate fat mass, such as IL-15, influence lymphocytes within adipose tissue and affect adipocyte size and fat mass through their activation. The effects of IL-15 on NK, NKT, and T cells include: to promote the development, survival, and activation of NK and NKT cells [13,25,26,34,35]; control the induction of $CD4^+$ memory T cells, as well as the survival and proliferation of activated naïve and memory $CD8^+$ T cells [26,27,34]; and induce interferon-gamma (IFN-γ) production, cytotoxicity, and perforin/granzyme expression in both NK cells and $CD8^+$ T cells and TNF-α in $CD8^+$ T cells [12,25,35,36]. Nevertheless, the actual function of these cell types in lean adipose tissue is unknown.

*In* vitro studies have shown that IL-15 directly affects adipose tissue using cultured adipocytes. Recombinant IL-15 treatment was shown to inhibit preadipocyte differentiation and lipid deposition using the murine adipogenic 3T3-L1 cell line [37]. Similar results were shown in lipoaspirate-derived human adipocytes treated with IL-15 at time of differentiation [14]. Inhibition of preadipocyte differentiation is associated with increased mRNA expression of calcineurin [38] and/or alterations in Signal transducers and activator of transcription 5 (STAT5) expression [39]. As well, IL-15 administration has been recently shown to affect lipid content in mature differentiated adipocytes *in vitro*, suggesting that IL-15 directly affects adipocytes independent of lymphocytes [39].

It has been previously shown, using ribonuclease protection assays, that mRNA for all three IL-15 receptor signaling subunits (α, β, γ) are present in white adipose tissue, suggesting that circulating IL-15 is capable of directly signaling in adipocytes [18]. IL-15 signals through a heterotrimeric receptor complex, including a β subunit shared with IL-2 and a γ-chain subunit shared with other interleukins (IL-2, -4, -7, -9, and -21). The third unique α subunit confers specificity and high affinity binding of IL-15 to this complex [40,41]. Studies have shown that IL-15 signals differently in immune lymphocytes compared to myleiod populations [42,43, Reviewed in 41]. The mechanism of IL-15 signaling in adipocytes is currently unknown.

The lymphocyte deficient RAG2$^{-/-}\gamma_c^{-/-}$ mouse model lacks mature NK cells due to the absence of the receptor γ-chain subunit, which is required for NK cell maturation. The importance of signaling through the receptor γ-chain has been previously examined in other animal models. Alvarez *et al.* showed IL-15 treatment reduced white adipose tissue mass without altering food intake in leptin-deficient ob/ob mice but not in leptin receptor-negative fa/fa Zucker rats. These authors determined that IL-15 did not effect adipose tissue in these obese rats due to a down regulation in receptor γ-chain expression [18]. Our data in RAG2$^{-/-}\gamma_c^{-/-}$ mice, on the other hand, demonstrates that the receptor γ-chain is not required for IL-15 to induce weight loss and reduce adipocyte cell size. This suggests that the γ-chain receptor subunit is dispensable for IL-15 signaling in adipocytes or compensatory mechanisms may occur in its absence. Although the

receptor γ-chain has been shown to be indispensible for NK and NKT cell development [32,44], we have previously shown that IL-15 may mediate its effects in the absence of this receptor subunit resulting in anti-tumor and anti-viral activity [28,45], as well as in activating myeloid immune cells [46].

Since IL-15 has been shown to decrease white adipose mass [19], we believe that acute IL-15 administration may result in direct lipolysis of adipose tissue, resulting in the release of free fatty acids into circulation. Alemendro *et al.* [22] previously showed that the administration of IL-15 in rats resulted in increased whole body and skeletal muscle fatty acid oxidation. Increased mRNA expression of several genes involved in fatty acid oxidation including carnitine palmitoyltransferase II (CPT-II) and peroxiome proliferator-activated receptor- δ (PPAR-δ) in the liver and skeletal muscle, respectively, demonstrates that IL-15 may induce the breakdown of fatty acids at peripheral tissues. Whether IL-15 treatment promotes altered mitochondrial function by increasing fatty acid usage in the liver and skeletal muscle needs to be determined. Another possible mechanism in which IL-15 may exert its weight loss effects is through the activation of myeloid derived cells. Interleukin-15 expression has been reported to alter macrophage activity in IL-15 tg and IL-$15^{-/-}$ mouse models [28,47]. Adipose tissue macrophages have been shown to alter insulin sensitivity in adipose tissue [1]. Recently, M2 polarized macrophages have been found to directly induce lipolysis through the release of catecholamines in white adipose tissue [48]. Whether or not IL-15 treatment influences macrophage polarization and possible secretion of lipolytic factors within white adipose tissue has yet to be examined. Lastly, rats given a single intravenous injection of this cytokine had a significant decrease in triglyceride absorption, without affecting gastric emptying and intestinal mobility [49]. Therefore, altered intestinal lipid absorption may in part explain the anti-obesity effects of IL-15.

In conclusion, our data suggest that IL-15 treatment results in an accumulation of NK, NKT, and CD3 T lymphocytes in adipose tissue. However, IL-15 mediates weight loss independent of lymphocyte activation and signaling through the common γ-chain. There is currently a lack of knowledge regarding the effects of IL-15 on metabolic tissues. Our findings have clear implications in the field of immuno-metabolism by demonstrating the importance of immune factors, like IL-15, in regulating adipose tissue mass. Future studies should continue to focus on determining the mechanism(s) in which IL-15 affects adipocytes.

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## Author Contributions

Conceived and designed the experiments: AAA. Performed the experiments: NB MC SR. Analyzed the data: NB. Wrote the paper: NB AAA.

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## CHAPTER 4

### Interleukin-15 modulates adipose tissue by altering mitochondrial mass and activity.

Nicole G. Barra, Rengasamy Palanivel, Emmanuel Denou, Marianne V. Chew, Amy Gillgrass, Tina D. Walker, Josh Kong, Carl D. Richards, Stephen M. Collins, Bernardo L. Trigatti, Alison C. Holloway, Sandeep Raha, Gregory R. Steinberg, and Ali A. Ashkar

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This manuscript examined the underlying mechanism(s) mediating differences in body weight between our three strains of mice. We found differences in body weight occurred independently of food intake, activity, and macronutrient absorption. IL-15tg mice had increased mitochondrial activity and mass specific to adipose tissue, compared to IL-15-/- and B6 mice and acute IL-15 administration induced the expression of adipose tissue markers associated with FAO. Lastly, IL-15 can directly affect adipocytes by promoting increased mitochondrial membrane potential and decreased lipid deposition.

Dr. Ali Ashkar and I were responsible for the design and interpretation of the experiments. Rengasamy Palanivel, Emmanuel Denou, Marianne V. Chew, and I were responsible for the generation of the data. Amy Gillgrass genotyped and supplied IL-15tg mice, while Tina D. Walker and Josh Kong inserted temperature probes in the visceral cavity of mice. I wrote the manuscript and all other members supplied reagents and contributed to the editing of this manuscript.

**Interleukin-15 modulates adipose tissue by altering mitochondrial mass and activity.**

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Key Words: obesity, interleukin-15, adipocytes, adipose tissue, gut microbiota, intestinal absorption, mitochondria, fatty acid oxidation

**ABSTRACT**

Interleukin-15 (IL-15) is an immunomodulatory cytokine that affects body mass regulation independent of lymphocytes; however, the underlying mechanism(s) involved remains unknown. In an effort to investigate these mechanisms, we performed metabolic cage studies, assessed intestinal bacterial diversity and macronutrient absorption, and examined adipose mitochondrial activity in cultured adipocytes and in lean IL-15 transgenic (IL-15tg), overweight IL-15 deficient (IL-15-/-), and control C57Bl/6 (B6) mice. Here we show that differences in body weight are not the result of differential activity level, food intake, or energy expenditure. Although intestinal microbiota differences between obese and lean individuals are known to impact macronutrient absorption, differing gut bacteria profiles in these murine strains does not translate to differences in body weight in colonized germ free animals and macronutrient absorption. Due to its contribution to body weight variation, we examined mitochondrial factors and found that IL-15 treatment in cultured adipocytes resulted in increased mitochondrial membrane potential and decreased lipid deposition. Lastly, IL-15tg mice have significantly elevated mitochondrial activity and mass in adipose tissue compared to B6 and IL-15-/- mice. Altogether, these results suggest that IL-15 is involved in adipose tissue regulation and linked to altered mitochondrial function.

## INTRODUCTION

Current global obesity statistics are staggering - age-standardized prevalence has nearly doubled from 6.4% in 1980 to 12.0% in 2008, amounting to over 600 million individuals classified as obese (1). This international epidemic is especially alarming not only due to the unprecedented rate by which incidence of obesity is increasing, but because obesity leads to the development of numerous chronic conditions such as diabetes, heart disease, osteoarthritis, mental illness, sleep apnea, and some forms of cancer (1-3). With rising rates of childhood obesity and its associated co-morbidities, obesity represents a tremendous burden on the health of succeeding generations (3-5). Therefore, understanding underlying causes and mechanisms of this disease is critical for preventing obesity from continuing to overwhelm international health care systems.

Simply monitoring an individual's caloric intake and/or activity level is not an effective treatment strategy for obesity since a variety of factors may contribute to the manifestation of this disease (6). Intriguing research has recently emerged examining the role of intestinal microorganisms in energy homeostasis and obesity. Variations in gut bacterial diversity exist between obese and lean individuals, suggesting differences in digestion and absorption could be important factors in the pathogenesis of obesity (7-9). Reports have shown that obese humans, diet induced obese and genetically obese mice have significantly fewer Bacteriodetes and an increase in Firmicutes phyla compared to lean controls (7-9). Research has shown that early differences in gut

microbiota in children followed from the first year to 7 years of life correlates with an overweight or obese status (10). Lastly, dietary weight loss in humans correlates with altered gut flora, characterized by an increase in Bacteriodetes and decrease in Firmicutes phyla (9). These reports suggest strategies targeting the modulation of the gut microbiota may provide unique treatments for obesity. These findings challenge the classical understanding of the causes of obesity and make obvious the need to better understand the relationship between the gut microbiota and obesity.

Interleukin-15 (IL-15) is an immunomodulatory cytokine primarily known for its effects on lymphocytes, specifically Natural Killer (NK) cells and CD8+ T cells (11-13), and more recently, for its association with obesity and body mass regulation. It has been reported that obese rodents and individuals have lower circulating levels of this cytokine (14-16) and treatment with IL-15 induces weight loss in wildtype C57BL/6 (B6) mice (17), IL-15-/- mice, and murine models of obesity (16, 18). IL-15 has been shown to decrease adiposity, lipid incorporation into adipose tissue, and adipocyte size (16, 17, 19-22), as well as affect adipokine adiponectin secretion (23). Since immune cells may directly induce lipolysis in white adipose tissue (24) and IL-15 affects the survival and activity of lymphocytes (11-13), we previously examined the nature of these interactions and found that IL-15 mediated weight loss occurs independent of lymphocytes (17). Uncovering the potential lipolytic effects of IL-15 on adipose tissue therefore requires examining its potential effects on mitochondrial fatty acid usage. Interestingly, recent

reports suggest that IL-15 treatment affects mitochondrial function by increasing the expression of mitochondrial enzymes such as carnitine palmitoyltransferase-1α (*CPT-1α*), which is involved in fatty acid oxidation, in both brown adipose tissue and in CD8+ memory T cells, as well as promotes mitochondrial biogenesis in this T cell subset (21, 25). As well, IL-15 treatment has been shown to induce uncoupling protein-1 expression in brown adipose tissue (21), skeletal muscle expression of peroxisome proliferator-activated receptor (PPAR)-δ, and significantly increase whole body and skeletal muscle fatty acid oxidation, while decreasing muscle fat content (22). Altogether, these results suggest that IL-15 treatment may have direct effects on mitochondrial factors involved in beta oxidation in adipocytes. Since IL-15 has been reported as an anabolic factor for skeletal muscle (26), understanding how IL-15 may contribute to the regulation of body weight, adipose tissue, and adipocyte size may reveal novel molecular pathway(s) involved in altering body composition.

Another recently described observation that may contribute to the obesogenic state is mitochondrial dysfunction in host metabolic tissues. Mitochondria are organelles within nucleated cells involved in producing energy in the form of adenosine triphosphate (ATP) molecules through the oxidation of macronutrients including fatty acids. It is believed that the majority of the body's daily ATP needs are produced by beta oxidation of fatty acids rather than carbohydrates (27). Recent reports have also suggested that increases in body weight and the obesity that results may be the

outcome of mitochondrial dysfunction and altered oxidative capacities (27-31). Decreased mitochondrial mass, activity and DNA (mtDNA) copy number have been reported in murine ob/ob, db/db, and diet induced obesity models (32, 33). Negative correlation between degree/severity of obesity and expression and activity of mitochondrial oxidative phosphorylation (OXPHOS) components have also been observed in human subjects (34). Lastly, altering mitochondrial activity has been shown to protect mice against diet-induced obesity (35). Improved understanding of the multiple factors that may contribute to alterations in body weight and obesity development, including the gut microbiota and mitochondrial activity may offer novel insights into key alternative strategies for the treatment of obesity and its associated co-morbidities.

In this study, we sought to determine the underlying mechanisms involved in mediating differences in body weight between IL-15tg, B6, and IL-15-/- mice. We first determined if varying levels of IL-15 expression altered activity level, food consumption, and energy expenditure in our three murine strains by performing metabolic cage studies. In order to examine the role of the intestinal microbiota in mediating differences in weight, we assessed intestinal bacterial diversity, performed colonization experiments using germ free animals, and evaluated intestinal macronutrient absorption with a glucose and lipid gavage. To determine whether IL-15 has direct effects on adipocytes, we treated mature murine 3T3-L1 adipocytes and assessed its lipolytic

effects, genetic expression of mitochondrial fatty acid oxidation markers, and adipokine secretion. Lastly, we examined mitochondrial factors in cultured adipocytes and in metabolic tissues of our three strains of mice. Altogether, these experiments will contribute to our understanding of the underlying mechanisms involved in IL-15's effects on body weight and its regulation of adipose tissue.

## RESULTS

### Varying expression of IL-15 does not alter food consumption, water intake, activity level, energy expenditure, and core body temperature.

To investigate the underlying mechanisms by which IL-15 regulates body mass, we performed metabolic cage studies in two month old female mice prior to differences in body weight among strains. At this time point, there were no significant differences in food intake, water intake, and activity level between IL- 15tg, IL-15-/-, and B6 mice during the day and night cycles (**Figures 1A-C**). Analysis of respiratory gases showed no differences in respiratory exchange ratios (RER) between our three strains, suggesting similar utilization of macronutrients (**Figure 1D**). Lastly, there were no significant differences detected in core body temperature among the three strains of mice during the day and night cycles (**Figures 1E-G**).

### Varying expression of IL-15 alters gut microbiota, but does not alter glucose or lipid absorption.

Since striking variations in gut bacterial diversity exists between obese and lean individuals, (7-9), we wanted to determine whether differences in the gut microbiota exist among our three strains of mice. Using 16S DNA sequencing from collected stool samples, we determined that varying IL-15 expression resulted in different gut bacterial diversity among our three strains (**Figures 2A and 2B**). In order to test whether these

alterations mediate differences in body weight, we co-housed germ free animals with either an IL-15tg or IL-15-/- female colonizer and monitored their body weight over time. We found that over time, although the weights of the colonizer's diverged, the body weights of germ free animals colonized with either IL-15tg or IL-15-/- microbiota did not differ significantly suggesting the gut microbiota does not play a role in mediating differences in body weight (**Figures 2C and 2D**).

Studies have shown that differences in bacterial diversity in the gut alter macronutrient absorption (36, 37). As well, since IL-15tg mice are susceptible to autoimmunity, inflammation in the small intestine of these animals may hinder macronutrient absorption and subsequent utilization (38). To further elucidate potential defects in glucose and fat absorption that could contribute to altered body weight, overnight fasted 2 and 6 month old female IL-15tg, B6, and IL-15-/- mice were orally administered glucose or a lipid load of olive oil normalized to their body weight. Glucose or lipids were administered at these ages to determine if significant differences in glucose or lipid absorption among strains were age dependent. Oral lipids were administered in the presence or absence of the lipase inhibitor tyloxapol. We found no differences in glucose (**Figures 3A and 3B**) and triglyceride (**Figures 3C-F**) levels at any time point in both 2 (**Figure S1**) and 6 month old mice, suggesting that varying levels of IL-15 do not affect intestinal glucose and lipid absorption.

**IL-15 has direct effects on adipocytes and production of IL-6 and KC**

Previously, we have shown that AdIL-15 treatment induced cell shrinkage of mature adipocytes in RAG2-/-$\gamma_c$-/- mice at day 8 post-treatment (17). Whether this is a direct effect of IL-15 has yet to be determined. We and others have shown that IL-15 affects lipid deposition in differentiating human and murine 3T3-L1 adipocytes (16, 23, 39); however, these experiments examine the effects of IL-15 on adipocytes during differentiation and do not assess its effects on mature adipocytes *in vitro*. Conflicting reports have yet to clarify whether IL-15 can directly mediate lipolysis in mature adipocytes (19, 23). Therefore we utilized the murine preadipocyte 3T3-L1 cell line to determine if IL-15 directly affects adipocytes. Once terminally differentiated at day 14, murine adipocytes were treated with 0ng/ml, 10ng/ml, 50ng/ml, 100ng/ml, or 250ng/ml of recombinant murine (rm) IL-15 protein in triplicate for 8 days with cell supernatants collected every two days. As shown in **figures 4A-E**, IL-15 treatment reduced oil red O staining in a dose dependent manner. When the percent of area stained was quantified, adipocytes treated with 100ng/ml and 250ng/ml rmIL-15 for 8 days had significantly lower 'red' density staining compared to untreated adipocytes (**Figure 4F**). Non-esterified free fatty acid (NEFA) concentrations were measured in cell supernatants as a marker of lipolysis due to rmIL-15 treatment. **Figure 4G** shows that IL-15 treatment did not result in increased concentrations of NEFAs in culture supernatants compared to controls at any time point. We also wanted to determine whether short-term rmIL-15

treatment on adipocytes affected adipokine secretion. RANTES, TNF-α, and leptin levels were undetectable. Despite no significant difference in adiponectin production with cytokine treatment (**Figure 4H**), we found that after 24 hour treatment at various doses, mature adipocytes produce murine IL-6 (**Figure 4I**) at the highest IL-15 dose and keratinocyte-derived chemokine (mKC) in a dose-dependent fashion (**Figure 4J**). We then examined IL-6 and KC production over the 8 day time course and found that cytokine treatment induced KC production in a dose and time dependent manner (**Figure 4K**), while IL-6 was undetected from day 2 onwards (data not shown). Since IL-15 mediated weight loss in B6 mice (17), we then wanted to correlate this effect with KC and IL-6 *in vivo.* We found no significant difference in circulating KC and no detectable IL-6 among AdIL-15, AdControl, and PBS groups on day 8 (**Figure 4L**).

**IL-15 affects adipose mitochondrial membrane potential *in vitro* and mitochondrial mass and activity *in vivo*.**

Since differences in body weight have been attributed to altered mitochondrial function, we wanted to determine if IL-15 treatment resulted in altered mitochondrial activity *in vitro* by staining murine adipocytes with Mitotracker. Mitotracker is a mitochondria-specific cationic fluorescent dye used to evaluate mitochondrial membrane potential, which is important for the conversion of ADP-ATP via ATP synthase. When mature 3T3-L1 cells treated with 250ng/ml rmIL-15 for 24 hours were incubated with Mitotracker,

we found that mitochondrial membrane potential was significantly increased compared to control untreated adipocytes (**Figure 5**). These results suggest that IL-15 has direct effects on mitochondria in adipose tissue. We then wanted to assess IL-15's effects on genes associated with fatty acid oxidation. **Figure S2** shows genes that were upregulated and downregulated with rmIL-15 treatment *in vitro*. Significant genetic downregulation of genes involved in fatty acid catabolism was seen in acyl-coA dehydrogenase (*Acadsb*), oxidase (*Acox2*), and synthetase (*Ascl3*). *In vivo* analysis shows that AdIL-15 treated RAG2-/-$\gamma_c$-/- mice had an upregulation of mitochondrial fatty acid oxidation markers *CPT1a* and beta-hydroxyacyl CoA dehydrogenase (*CHAD*) in adipose tissue (**Figures 6A and 6B**). Altogether these results suggest that IL-15 treatment may promote fatty acid oxidation in adipose tissue.

Lastly, we wanted to determine if varying IL-15 expression results in altered mitochondrial function and mass within metabolic tissues of our three strains of mice. Livers, quadriceps muscles, and gonadal fat sources were excised from 2 and 6 month old IL-15tg, B6, and IL-15-/- female mice to determine complex IV (COX) activity, as a measure of mitochondrial activity, and for citrate synthase activity, as a measure of mitochondrial mass (40, 41). We found that 6 month old, but not 2 month old (**Figures 7A and 7B**) IL-15tg mice have significantly elevated mitochondrial activity and mass specific to adipose tissue compared to age-matched B6 and IL-15-/- mice (**Figures 7C and**

**7D**). Statistical analysis using two way ANOVA revealed no significant interaction between age and murine strain for adipose citrate synthase and COX measurements. These differences were not seen in liver or quadriceps muscle samples at either age (**Figures S3 and S4**). This data suggest that over time, overexpression of IL-15 results in increased mitochondrial activity and mass in adipose tissue.

**DISCUSSION**

Results from this study show that differences in body weight between lean IL-15tg, B6 and overweight IL-15-/- mice are not due to alterations in activity level, food intake, or energy expenditure. We also discovered that despite vast differences in the gut microbiota profiles among these murine strains, these differences did not affect glucose and lipid absorption, as well as differences in body weight over time in colonized germ free animals. *In vitro* studies using cultured 3T3-L1 adipocytes, demonstrate that IL-15 treatment stimulated the secretion of IL-6 and keratinocyte-derived chemokine (KC) in a dose and time dependent manner, as well as resulted in decreased lipid deposition over time. Lastly, IL-15 treatment in cultured adipocytes resulted in increased mitochondrial membrane potential *in vitro*. *In vivo*, IL-15 administration resulted in increased expression of fatty acid oxidation markers *CPT1a* and *CHAD*, while IL-15tg mice have significantly elevated mitochondrial activity and mass in adipose tissue compared to B6 and IL-15-/- mice.

Previous reports have examined the effect of IL-15 expression on host metabolism. Animal monitoring analyses have been recently conducted in a different, muscle specific murine IL-15tg mouse model (42). Similar to our previous report (16), the outcome in this model was significant reductions in body fat (20). Here, researchers found that four month old male IL-15tg mice had significantly lower RER and elevated total and ambulatory activity and energy expenditure compared to B6 control mice.

Similar to our results, however, no significant difference in food consumption was found (42). Unlike the transgenic model examined in this paper, the data from the muscle specific model suggest that overexpression of IL-15 correlated to increased fatty acid utilization and affects whole body energy expenditure and movement. The effects of cytokine overexpression on body composition have been previously reported to be dependent on gender. When comparing male and female IL-15tg mice, females had increased lean muscle mass compared to males, while male mice remained lean when challenged with a high fat diet unlike female mice, who had increased adiposity (20). These data suggest that gender effects may be evident when examining the metabolic consequences of varying IL-15 expression. Aside from differences in the age and gender of mice tested, substantial variation in the genetic regulation of and circulating IL-15 levels among the transgenic models may also contribute to the dissimilarity in results. With the IL-15tg mouse model utilized here, mature murine IL-15 peptide expression is under the control of the MHC class I $D^d$ promoter, with serum IL-15 measured at approximately 187pg/ml (43). In the mouse model examined by Quinn *et al*., cytokine expression is driven by the human alpha skeletal actin promoter and the transgene's signal sequence was replaced with that of IL-2, with circulating levels measured at approximately 103,000 pg/ml (20, 42). Therefore, examining the metabolic consequences of IL-15 overexpression will most likely depend on its genetic regulation, with differences in measured outcomes partially due to global or tissue specific

expression, as well as variation in circulating cytokine levels and gender of animals tested.

Along with human studies connecting altered gut microbiota to obesity, it has been previously reported that deficiencies in innate immune factors, such as toll-like receptor-5 (TLR-5), have been associated with significant alterations in gut microbiota and adiposity (44). Therefore, we wanted to determine if varying IL-15 expression correlated with differences in gut microbiota, and whether this mediated differences in body weight. Although we did not sequence the bacterial species characteristic of each mouse strain, we are the first group to show that varying IL-15 expression results in striking differences in the gut bacterial profile of mice. Significant alterations in the gut microbiota have been shown to affect body weight and other metabolic processes such as bile-acid metabolism and subsequent intestinal lipid absorption, insulin sensitivity, and hepatic steatosis (45-49). In our studies we found that body weight and intestinal lipid and glucose absorption did not differ in our three strains of mice, suggesting that the gut microbiota does not play a role in mediating differences in body weight or affect absorption. The precise role and biological significance of the unique gut bacterial profiles in our three strains of mice is unknown. These bacterial differences in mice with various IL-15 expression levels may exist as a consequence of the disparities in the intestinal immune and inflammatory status, due to the influence of IL-15. Another possibility is that since the gut microbiota is a rich source of molecules that may cause

inflammation, like lipopolyssaccharide and peptidoglycan (50), differences in the bacteria present may play a role in contributing to the intestinal inflammatory environment and/or gut permeability via effects on the gut epithelium. Research has shown that IL-15 expression plays a role in mediating intestinal inflammation in models of celiac disease and Crohn's disease (38, 51-53), suggesting a possible link between IL-15, gut microbiota, and intestinal inflammation. Defining the metabolic contribution of the gut microbiota in our three strains of mice is currently under investigation.

The results of this paper demonstrate that IL-15 has direct effects on adipocytes *in* vitro through the secretion of adipokines and reduction in adipocyte lipid content. The secretion of proinflammatory adipokines IL-6 and KC reported here, and of the anti-inflammatory adipokine adiponectin reported by Quinn *et al*. (23) by adipocytes after 4-6 days of treatment, may affect the inflammatory status of adipose tissue by IL-15 administration *in vivo*. Similar to Quinn's results, we did not find any significant adiponectin production within the first 24 hours of IL-15 treatment (23). Due to IL-6's known lipolytic effects (54), we wanted to determine how IL-6 production correlated with IL-15 treatment *in vivo* and *in vitro*. These results suggest that IL-6 may play a role in IL-15 stimulated lipolysis. The use of neutralizing antibodies against IL-6 would determine whether IL-15 requires IL-6 expression to induce weight loss *in vivo* and its effects on cultured adipocytes. In regards to the induction of KC production, at this time the authors are not aware of any published findings demonstrating this chemokine's

possible lipolytic stimulating properties. Murine KC is the homolog to human IL-8. Increased KC expression is found in adipose and plasma of ob/ob and diet induced obese mouse models, and is primarily produced by stromal vascular cells. Interestingly, KC is highly expressed by preadipocytes and decreases during adipogenesis. Although KC has no effect on adipogenesis, whether this chemokine has effects on mature adipocytes has yet to be determined (55).

In this study, we are also the first to show that IL-15tg mice have increased mitochondrial activity and mass specific to adipose tissue at 6 months of age compared to B6 and IL-15-/- mice. Whether these mitochondrial alterations precede and mediate changes in body weight, or result from body weight differences have yet to be determined. At the 2 month time point (**Figures 7A and 7B**), mitochondrial activity and mass appear to be slightly elevated in the adipose tissue of IL-15tg mice, suggesting mitochondrial alterations may precede differences in body weight. Previous reports suggest that deficiencies in IL-15 signaling (IL-15Rα-/- mice) increased skeletal muscle mitochondrial biogenesis, density, expression of complexes involved in the electron transport chain, and mitochondrial DNA content (56, 57). As well, muscle specific male IL-15tg mice had increased mRNA and protein expression of factors involved in promoting lipid oxidation in extensor digitorum longus muscles compared to control mice. The increased expression of skeletal muscle oxidation markers in muscle specific IL-15tg mice correlated to a significant increase in the duration of a single run-to-

exhaustion endurance test compared to B6 controls (42). However, we found no significant differences in quadriceps muscle mitochondrial mass and activity at either time point between IL-15-/-, B6, and IL-15tg mice.

We also show that IL-15 treatment increased mitochondrial membrane potential in cultured adipocytes when stained with Mitotracker. Since IL-15 treatment caused significant reduction in lipid content over time, we wanted to determine if this increase in mitochondrial membrane potential correlated to the induction of fatty acid oxidation by examining various genetic markers involved in this process. Whether this increase in mitochondrial membrane potential is specific to fatty acid oxidation is not certain in our *in vitro* model since no significant upregulation in these markers was found after 24 hours of treatment (**Figure S2**). However, our *in vivo* analysis demonstrates that IL-15 increases genetic expression of fatty acid oxidation markers such as *CPT1a*, an enzyme involved in mitochondrial fatty acid transport, and *CHAD*, an enzyme involved in beta oxidation. Similarly, other reports have shown that IL-15 administration resulted in increased *CPT1a* expression in brown adipose tissue and in CD8+ memory T cells (21, 25).

In order to produce adenosine-5'-triphosphate (ATP), breakdown of pyruvate and fatty acids must occur in the mitochondria through glycolysis and the Krebs cycle. Reduced intermediates are then shuttled through the electron transport chain, which generates the majority of the cell's ATP. The transport of electrons results in a potential

gradient across the mitochondrial inner membrane, which is essential for protons to enter the mitochondrial matrix resulting in ATP production (58). Since IL-15 treatment affects mitochondrial membrane potential, this suggests that cytokine treatment could directly affect the potential gradient across the mitochondrial inner membrane and therefore the electron transport chain, subsequently affecting ATP synthase activity and ATP production. Determining intracellular ATP production in adipocytes treated with IL-15 will determine whether IL-15 affects ATP production. Altogether, this suggests that IL-15 mediates its effects directly on adipocytes through the induction of lipolysis and subsequent oxidation of fatty acids, leading to increased ATP production.

In summary, our findings suggest that IL-15 directly affects adipocytes through adipokine secretion of KC and IL-6, reduction in lipid content, and increases mitochondrial membrane potential *in vitro*. Our animal studies suggest that IL-15 expression alters mitochondrial activity and mass and induces genetic expression of fatty acid oxidation markers *in vivo*, which could modulate body composition. Further work is needed in order to define the biochemical and signaling mechanism(s) of IL-15 action on adipose tissue, as well as its effects on other metabolic tissues to mediate these alterations in body composition. Similar to the approach of this paper, examining a variety of factors that may alter body weight is necessary in order to thoroughly understand the importance and relative contribution of each, which will lead to improved therapeutic and preventive strategies for obesity.

## METHODS

### Mice

A breeding pair of IL-15tg mice on a C57BL/6 background was kindly provided by M. Caligiuri (Ohio State University, School of Medicine, Columbus, OH), while breeding pairs of C57BL/6 and IL-15-/- mice were purchased from Charles River Laboratory (Montreal, Quebec, Canada) and Taconic (Germantown, NY, USA), respectively. Female lymphocyte deficient Balb/c RAG2-/-$\gamma_c$-/- mice were bred from breeding pairs given as a gift by M. Ito (Central Institute for Experimental Animals, Kawasaki, Japan). These three strains of mice were bred and maintained at McMaster University's Central Animal Facility. All experiments were approved by the Animal Research Ethics Committee. Mice were housed in level B rooms and maintained under controlled lighting (12 hour light/12 hour dark cycle) and temperature (22°C) with ad libitum access to water and a low 5% fat chow diet. At 2 months of age, metabolic monitoring was performed using a Comprehensive Lab Animal Monitoring System (Columbus Instruments, Columbus, OH) as previously described (59). Core body temperature was assessed by visceral implantation of TA-F10 mouse temperature transmitters and analyzed using ART v4.3 software (Data Sciences International, St. Paul, Minnesota) over a 72 hour period.

**Bacterial DNA Determination of Gut Microbiota**

Bacterial DNA was extracted from stool samples collected from 2 month old female IL-15tg, B6, and IL-15-/- mice using the QIAamp DNA Stool Mini Kit (QIAGEN, Toronto, Ontario, Canada) and quantified spectrophotometrically. Amplification of the hypervariable V3 region from the bacterial 16S ribosomal DNA using polymerase chain reaction was completed with universal bacterial primers (HDA1-GC,HDA-2; Mobixlab, McMaster University core facility, Hamilton, Ontario, Canada) as previously described (60). Denaturing gradient gel electrophoresis (DGGE) was performed using a DCode universal mutation system (Bio-Rad, Mississauga, Ontario, Canada) and the electrophoresis was conducted at 130 V, 60°C for 4.5 hours. Gels were stained with SYBR green I (Sigma, St. Louis, MO, USA) and viewed by ultraviolet transillumination. A scanned image of the electrophoretic gel was used to measure the staining intensity of the fragments using Quantity One software generating an electrophoregram (version 4-2; Bio-Rad Laboratories). The staining intensity of each fragment was compared using the Dice similarity coefficient (60).

Two month old female germ-free mice acquired from McMaster University's gnotobiotic unit, were co-housed with either a two month old female IL-15tg or IL-15-/- mouse and their body weights were monitored on a weekly basis during a 6 month period.

**Glucose and Triglyceride Gavage**

At both 2 and 6 months of age, female IL-15tg, B6, and IL-15-/- mice were administered 2 g/kg D-glucose or 10µl/g olive oil following an overnight fast. The olive oil gavage was performed in either the absence or presence of the lipase inhibitor tyoxapol (Sigma, St. Louis, MO, USA), where mice were administered 0.5mg/g intravenously immediately after gavage. Blood samples were collected by submandibular bleeds at baseline (time 0) and at various time points post-gavage from each mouse per group. Blood glucose was measured using a hand-held glucometer (Accu-Chek Active, Roche Diagnostics, Laval, Canada). Following the olive oil gavage, blood was passed through heparinized micro-hematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA, USA), collected in eppendorf tubes, and centrifuged at 12,000 rpm for 10 minutes at 4°C to collect plasma. Triglyceride plasma levels were measured using the L-type triglyceride M colorimetric assay (Wako Diagnostics, Richmond, VA, USA). The area under the curve for the total glucose and triglyceride responses was assessed using the trapezoidal rule. Linear regression analysis was used to calculate slopes for triglyceride responses in tyoxapol treated mice.

**Cell Culture**

Murine preadipocyte 3T3-L1 cells were purchased from ATCC and grown in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% bovine serum (Invitrogen,

Burlington, Ontario, Canada). Cells were seeded at 200,000 cells/well in a 24 well plate and grown/cultured until confluent. Twenty four hours later, confluent cells were treated with adipogenic induction media, defined as day 0, which includes 10% fetal bovine serum (FBS), 10µg/mL human insulin (Invitrogen, Burlington, Ontario, Canada), 250 nM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) in DMEM for 48 hours (Sigma, St. Louis, MO, USA). The cells were then given 10% FBS DMEM post-induction media for another 12 days. During this time, cells were given fresh media every 2-3 days. Once terminally differentiated at day 14, adipocytes were treated with 0ng/ml, 10ng/ml, 50ng/ml, 100ng/ml, or 250ng/ml of recombinant murine (rm) IL-15 protein (Peprotech, Rocky Hill, NJ, USA) in triplicate for 8 days with cell supernatants collected every two days. Non-esterified free fatty acid (NEFA) (Wako Diagnostics, Richmond, VA, USA) concentrations were quantified from collected cell supernatants. After 8 days of treatment (day 22), lipid droplets from treated and untreated adipocytes were stained with Oil Red O as previously described (23) and photographed. Images were then loaded into ImageJ v1.45s where the 'red' density area was approximated by varying the threshold tool. This area and the entire image section were measured and represented as the percentage of red staining from the total area.

**Delivery of IL-15**

Sixteen week old female B6 and RAG2-/-$\gamma_c$-/- mice were administered either 5 x$10^8$ pfu of the Ad-expressing human IL-15 vector Opt.hIL-15 ("AdIL-15"), an empty adenoviral vector ("AdControl"), or 200µl PBS via IV tail injections on days 0, 2, and 4 (n=5 per group) as previously described (61). On day 8, blood was collected from anaesthetized B6 mice through the abdominal aorta and centrifuged at 5,000 rpm for 10 minutes to collect serum. On day 8, adipose tissue was collected from RAG2-/-$\gamma_c$-/- mice, homogenized in TRIzol, and stored at -80° Celsius (Invitrogen, Burlington, Ontario, Canada) as per manufacturer's instructions.

**ELISAs for murine leptin, adiponectin, RANTES, TNFA, IL-6 and KC**

Cell supernatants were collected from differentiated 3T3-L1 adipocytes treated with 0ng/ml, 10ng/ml, 50ng/ml, 100ng/ml, and 250ng/ml of rmIL-15 for 24 hours and examined for murine leptin, adiponectin, regulated on activation normal T cell expressed and secreted (RANTES), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and keratinocyte-derived chemokine (KC) using DuoSet ELISA kits (R&D Systems, Minneapolis, MN, USA).

**Confocal Microscopy**

Murine 3T3-L1 cells were grown and differentiated into adipocytes on glass cover slips in a 24 well plate. At day 14, adipocytes were then treated with 0ng/ml or 250ng/ml rmIL-15 for 24 hours and incubated with 500nM of Mitotracker Red CMXRos (Invitrogen, Burlington, Ontario, Canada) for 30 minutes. Cells were then fixed with 4% PFA and counterstained for nuclei with propidium iodide and attached to microscope glass slides using Vectasheild hard-set mounting medium (Vector, Burlington, Ontario, Canada). Images were captured by LSM510 (inverted) confocal miscroscope (Zeiss, Oberkochen, Germany) under an x63 objective and analyzed using LSM 510 version 3.2 software (Zeiss, Oberkochen, Germany).

**Mitochondrial Enzyme Activity**

Livers, quadriceps muscles, and gonadal fat were excised from 2 and 6 month old IL-15tg, B6, and IL-15-/- female mice, flash frozen in liquid nitrogen, and stored at -80°C until homogenized. Tissue samples were homogenized in homogenization buffer (100mM KCl, 220mM mannitol, 70mM sucrose, 1mM EGTA, and 5mM HEPES pH 7.4), centrifuged at 600xg for 5 minutes, and supernatants were collected and used to determine complex IV activity, as a measure of mitochondrial activity, and for citrate synthase activity, as a measure of mitochondrial mass (40, 41). Both assays were performed as previously described using UV-spectrophotometry and the data presented

are expressed as the mean enzyme activity (nmol/min/mg protein) relative to the wet weight of tissue (40, 62, 63).

**Assays of mRNA Expression**

RNA was isolated from adipose tissue of AdIL-15, AdControl, or PBS treated RAG2-/-$\gamma_c$-/- mice on day 8, and from differentiated murine 3T3-L1 adipocytes treated with 0ng/ml or 500ng/ml rmIL-15 for 24 hours, purified on RNeasy spin columns (QIAGEN, Toronto, Ontario, Canada), and treated with deoxyribonuclease and genomic DNA removal buffer. Then, 400ng of RNA was reverse transcribed into cDNA ($RT^2$ First Stand Kit; QIAGEN, Toronto, Ontario, Canada). Real-time (RT)-PCR was performed using the $RT^2$ Fatty Acid Metabolism PCR array purchased from QIAGEN as per manufacturer's instructions using treated and nontreated 3T3-L1 adipocytes. Primer sets used for RT-PCR murine adipose tissue analysis include: *CPT1α* forward and reverse 5'-GCTGGGCTACTCAGAGGATG-3' and 5'-CACTGTAGCCTGGTGGGTTT-3'; and *CHAD* forward and reverse 5'-ACCAAACGGAAGACATCCTG-3' and 5'-AGCTCAGGGTCTTCTCCACA-3'. Preliminary experiments conducted by our lab have shown no statistical differences in the expression of these markers between AdControl and PBS treated tissues and are therefore combined as one group ("AdControl/PBS"). Cycle threshold ($C_T$) values were set identically for all plates, sample $C_T$ values were normalized to GAPDH $C_T$, and fold

change of gene expression between untreated controls and treated groups were calculated.

**Statistics**

Greater than two group comparisons were made using one-way ANOVA followed by Tukey's post hoc multiple comparisons test with Graph Pad Prism 4 software (La Jolla, CA, USA). Two-way ANOVA followed by Bonferroni post tests were used to determine possible strain and time effects on mitochondrial COX and CS measurements. Student t-test was used for two group comparisons. Results are expressed as mean ± SEM. To compare the intestinal microbiota, the similarity between DGGE profiles was determined using the Dice similarity coefficient and tested for significance using Quantity One software (Biorad, Mississauga, Ontario). Significance is indicated when $p<0.05$.

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**FIGURES**

**Figure 1: Varying expression of IL-15 does not alter food consumption, water intake, activity level, energy expenditure and core body temperature.** Bar graphs show **(A)** food consumption, **(B)** water intake, and **(C)** activity levels of 2 month old female IL-15tg (n=4), B6 (n=5), and IL-15-/- (n=5) mice individually housed in metabolic cages (Columbus Instruments Laboratory Animal Monitoring System) for 72 hours. **(D)** Energy expenditure is represented as a respiratory exchange ratio (RER) determined by $CO_2$ production and $O_2$ intake between 3 strains of mice. **(E)** Line graph shows average core body temperature during the day and night cycle of mice with visceral cavity temperature probes over a 72 hour period. Average **(F)** day and **(G)** night body temperatures depicted in bar graphs.

**Figure 2: Varying expression of IL-15 alters gut microbiota and does not play a role in modulating body weight. (A)** PCR-DGGE targeting the v3 (variable region #3) region from bacterial 16S rDNA sequences isolated from IL-15tg (n=5), B6 (n=9), and IL-15-/- mice stool samples (n=4). **(B)** Profile similarity generated from inter-mouse group comparisons (IL15-/- vs IL15tg vs B6). Intra-group comparisons were not significantly different from each other (i.e. mice within each strain). Body weights of **(C)** IL-15tg and IL-15-/- mice colonizers (n=1/group) and **(D)** colonized germ free C57Bl/6 (n=4/group) animals over 6 months. ***$p<0.001$

**Figure 3: Varying IL-15 expression does not alter glucose or lipid absorption. (A)** Blood glucose (mmol/l) and triglyceride (mg/dl) concentrations in the **(C)** absence or **(E)** presence of the lipase inhibitor tyoxapol at baseline and at various time points following administration of an oral glucose or olive oil load normalized to body weight in 6 month old female mice. Bar graphs depict area under the curve (AUC) or slope for the total **(B)** glucose and **(D, F)** lipid responses during the oral challenge. (n=5/group)

**Figure 4: IL-15 treatment results in decreased lipid deposition in 3T3-L1 mature adipocytes and IL-6 and KC production with increasing IL-15 treatment.** Mature 3T3-L1 adipocytes were treated every 2 days with either **(A)** 0, **(B)** 10, **(C)** 50, **(D)** 100, or **(E)** 250 ng/ml of recombinant murine IL-15 and were stained with Oil Red O. Pictures were taken under a ×10 objective. **(F)** Relative semi-quantitative differences in lipid deposition were confirmed using ImageJ software (n=3 images/group). **(G)** Non-esterified fatty acid concentrations were quantified in 3T3-L1 cell supernatants at each time point (n = 3/group). Differentiated 3T3-L1 adipocytes were seeded at $2x10^5$ cells/well and stimulated in triplicate with the indicated concentrations of rmIL-15 for 24 hours at 37°C. Differentiated 3T3-L1 adipocytes were stimulated in triplicate with the indicated concentrations of rmIL-15 for 24 hours at 37°C and cell supernatants were analyzed for **(H)** adiponectin, **(I)** interleukin-6 (IL-6), and **(J)** keratinocyte-derived chemokine (KC) production by ELISA. **p<0.01, ***p<0.001

**Figure 5: IL-15 treatment increases membrane potential in 3T3-L1 mature adipocytes *in vitro.*** Confocal images show fixed mature 3T3-L1 cells either **(A)** untreated or **(B)** treated with 250ng/ml rmIL-15 for 24 hours. Cells were stained with mitotracker (41) and counterstained with SYTO nucleic acid stain (green). Images were obtained using LSM 510 confocal microscope using x63 objectives.

**Figure 6: AdIL-15 treatment increases expression of fatty acid oxidation markers *CPT1a* and *CHAD* in adipose tissue.** RAG2-/-$\gamma_c$-/- mice were treated with either AdIL-15 or AdControl/PBS on days 0, 2, and 4. RNA was isolated from collected adipose tissue on day 8 and analyzed for genetic expression of **(A)** CPT1a and **(B)** CHAD using RT-PCR. Genetic mRNA expression levels were standardized to internal GAPDH levels and reported as fold increase relative to AdControl/PBS treated animals. (n=5/group) **P<0.01.

**Figure 7: IL-15tg mice have increased mitochondrial activity and mass at 6 months of age in adipose tissue.** Adipose tissue homogenates from **(A, B)** 2 and **(C,D)** 6 month old female IL-15tg, B6, and IL-15-/- mice were analyzed for **(A,C)** complex IV activity and **(B,D)** citrate synthase activity. Data are expressed as the mean enzyme activity (nmol/min/mg protein). (n=5 per group unless otherwise stated; for 6 months of age IL-15tg (n=4/group) and B6 (n=4/group)) *P<0.05, **P<0.01.

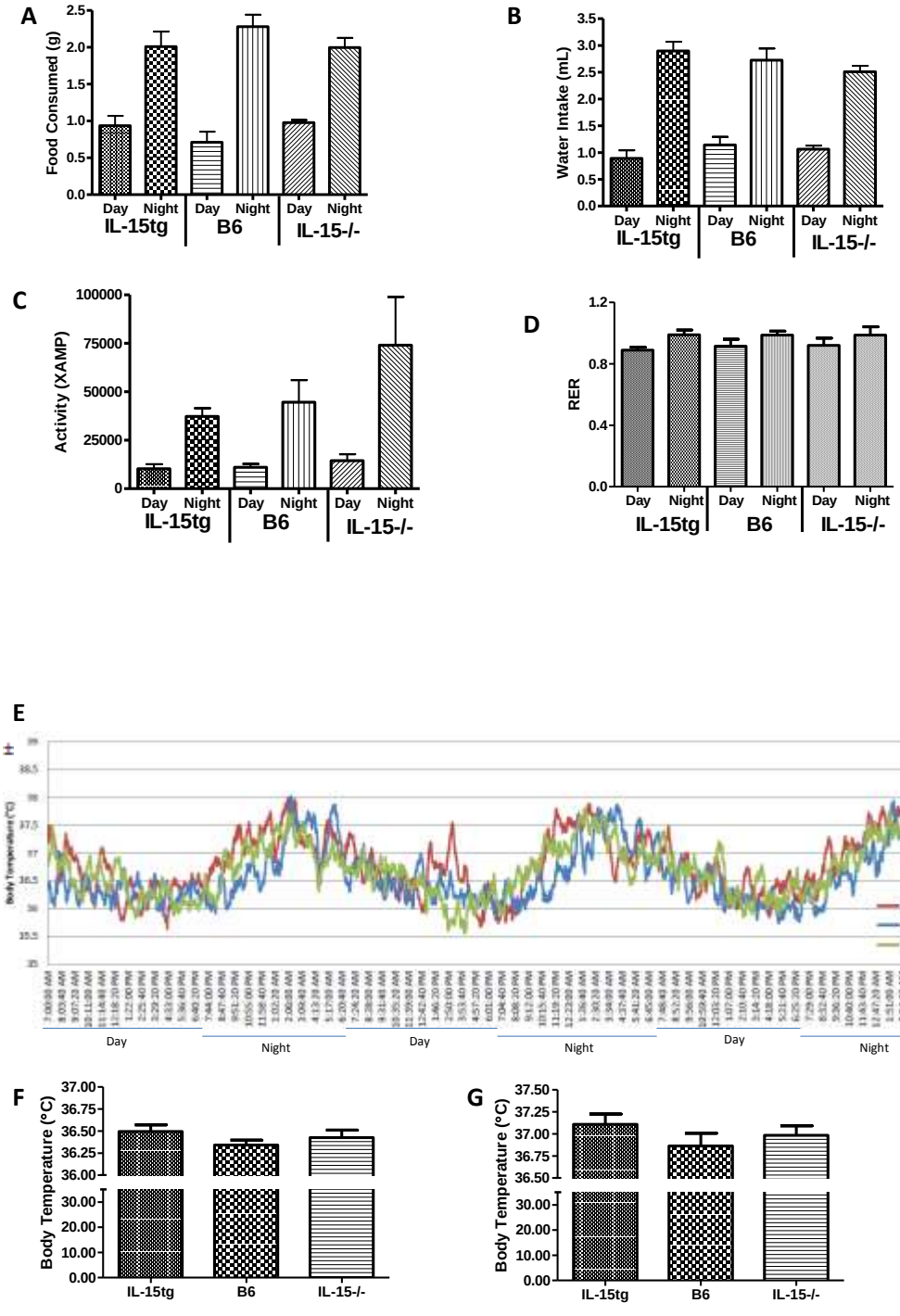
**Supplemental Figure 1: Varying IL-15 expression does not alter glucose or lipid absorption at 2 months of age. (A)** Blood glucose (mmol/l) and **(C)** triglyceride (mg/dl) concentrations at baseline and at various time points following administration of an oral glucose or olive oil load, normalized to body weight in 2 month old female mice. Bar graphs depict area under the curve (AUC) for the total **(B)** glucose and **(D)** lipid responses during the oral challenge. (n=5/group)

**Supplemental Figure 2: Acute IL-15 administration results in differential expression of fatty acid oxidation markers.** Comparative expression of fatty acid oxidation genes from mature 3T3-L1 adipocytes treated with 0ng/ml or 500ng/ml rmIL-15 for 24 hours normalized to GAPDH reveals a 1.2 fold difference or greater using PCR arrays (n=3/group).*P<0.05.

**Supplemental Figure 3: Mitochondrial activity and mass at 2 months of age in liver and quadriceps muscle. (A,C)** Liver and **(B, D)** quadriceps muscle homogenates from 2 month old female IL-15tg, B6, and IL-15-/- mice were analyzed for **(A,B)** complex IV activity and **(C,D)** citrate synthase activity. Data are expressed as the mean enzyme activity (nmol/min/mg protein). (n=5/group)

**Supplemental Figure 4: Mitochondrial activity and mass at 6 months of age in liver and quadriceps muscle. (A,C)** Liver and **(B, D)** quadriceps muscle homogenates from 6 month old female IL-15tg, B6, and IL-15-/- mice were analyzed for **(A,B)** complex IV activity and **(C,D)** citrate synthase activity. Data are expressed as the mean enzyme activity (nmol/min/mg protein). (IL-15tg and B6 (n=4/group), IL-15-/- (n=5/group)) *P<0.05

**Figure 1**


A
Food Consumed (g)
2.5
2.0
1.5
1.0
0.5
0.0
Day Night Day Night Day Night
IL-15tg B6 IL-15-/-
B
Water Intake (mL)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Day Night Day Night Day Night
IL-15tg B6 IL-15-/-
C
Activity (XAMP)
100000
75000
50000
25000
0
Day Night Day Night Day Night
IL-15tg B6 IL-15-/-
D
RER
1.2
0.8
0.4
0.0
Day Night Day Night Day Night
IL-15tg B6 IL-15-/-
E
Day
Night
Day
Night
Day
Night
F
Body Temperature (°C)
37.00
36.75
36.50
36.25
36.00
30.00
20.00
10.00
0.00
IL-15tg B6 IL-15-/-
G
Body Temperature (°C)
37.50
37.25
37.00
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30.00
20.00
10.00
0.00
IL-15tg B6 IL-15-/-

**Figure 2**

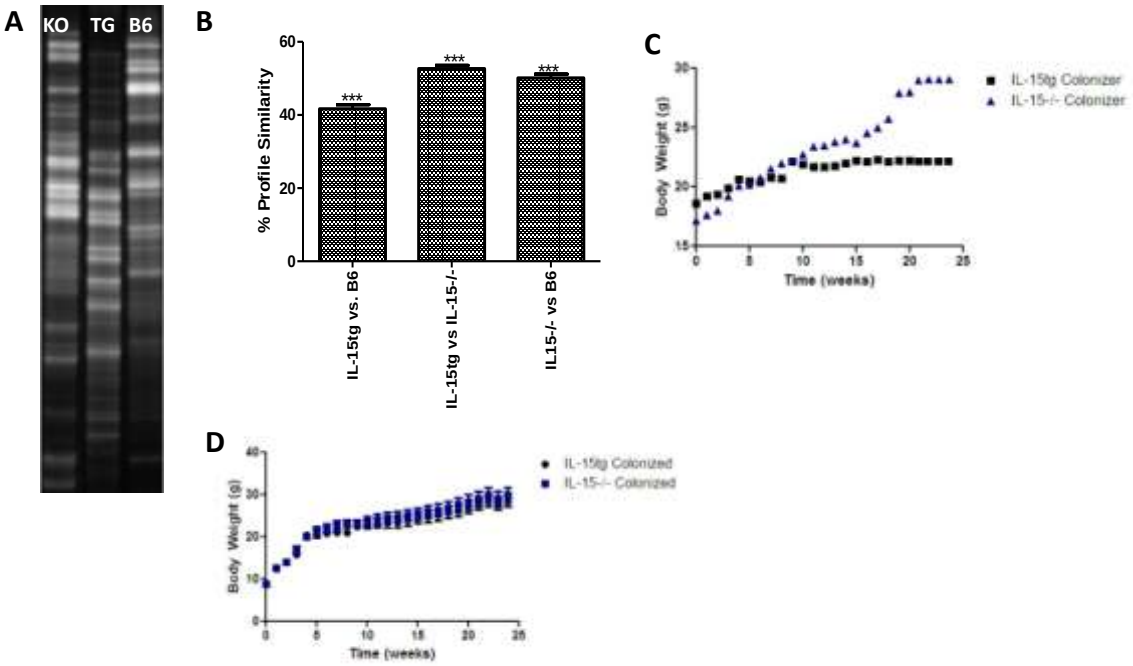

A
KO
TG
B6
B
% Profile Similarity
60
40
20
0
***
***
***
IL-15tg vs. B6
IL-15tg vs IL-15-/-
IL15-/- vs B6
C
Body Weight (g)
Time (weeks)
IL-15tg Colonizer
IL-15-/- Colonizer
D
Body Weight (g)
Time (weeks)
IL-15tg Colonized
IL-15-/- Colonized

**Figure 3**

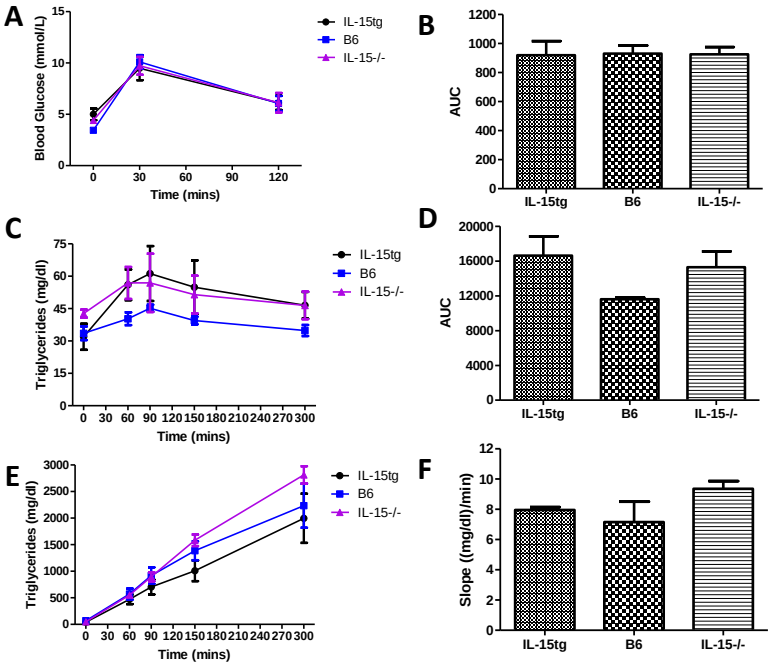

A
Blood Glucose (mmol/L)
Time (mins)
IL-15tg
B6
IL-15-/-
B
AUC
IL-15tg
B6
IL-15-/-
C
Triglycerides (mg/dl)
Time (mins)
IL-15tg
B6
IL-15-/-
D
AUC
IL-15tg
B6
IL-15-/-
E
Triglycerides (mg/dl)
Time (mins)
IL-15tg
B6
IL-15-/-
F
Slope ((mg/dl)/min)
IL-15tg
B6
IL-15-/-

**Figure 4**

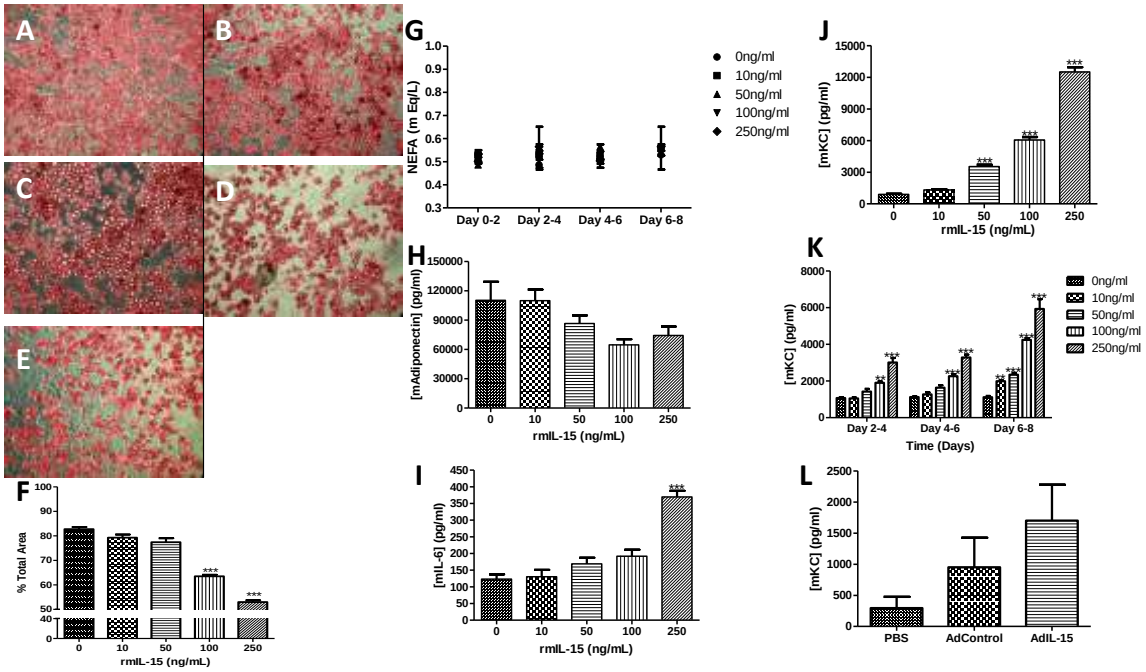

A
B
C
D
E
F
% Total Area
rmIL-15 (ng/mL)
G
NEFA (m Eq/L)
Day 0-2
Day 2-4
Day 4-6
Day 6-8
0ng/ml
10ng/ml
50ng/ml
100ng/ml
250ng/ml
H
[mAdiponectin] (pg/ml)
rmIL-15 (ng/mL)
I
[mIL-6] (pg/ml)
rmIL-15 (ng/mL)
J
[mKC] (pg/ml)
rmIL-15 (ng/mL)
K
[mKC] (pg/ml)
Time (Days)
L
[mKC] (pg/ml)
PBS
AdControl
AdIL-15

**Figure 5**

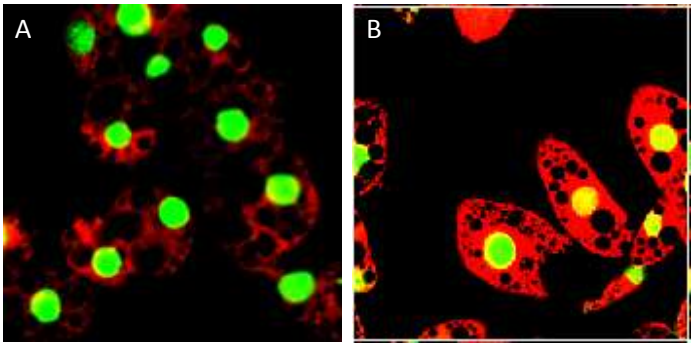


**Figure 6**

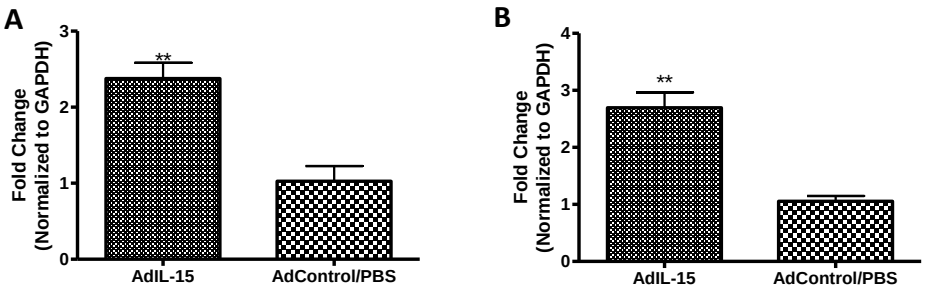
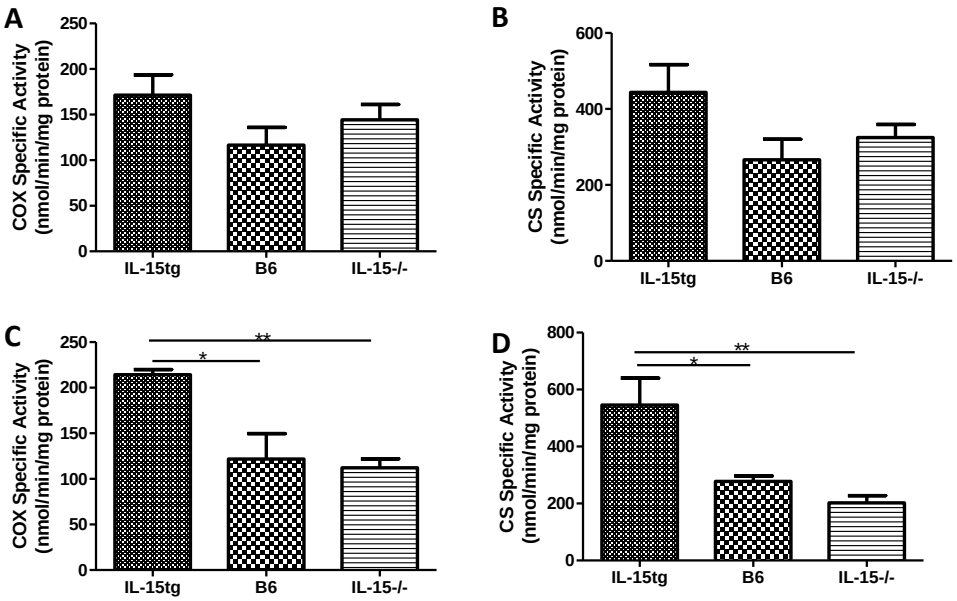
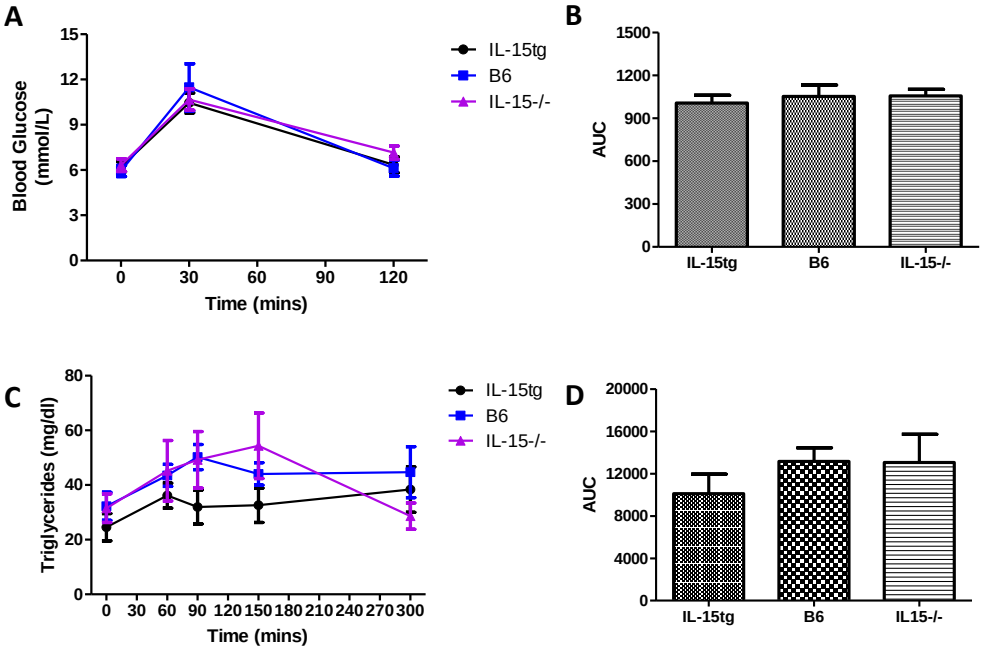

A
Fold Change
(Normalized to GAPDH)
3
2
1
0
**
AdIL-15
AdControl/PBS
B
Fold Change
(Normalized to GAPDH)
4
3
2
1
0
**
AdIL-15
AdControl/PBS

**Figure 7**


A
COX Specific Activity (nmol/min/mg protein)
250
200
150
100
50
0
IL-15tg
B6
IL-15-/-
B
CS Specific Activity (nmol/min/mg protein)
600
400
200
0
IL-15tg
B6
IL-15-/-
C
COX Specific Activity (nmol/min/mg protein)
250
200
150
100
50
0
**
*
IL-15tg
B6
IL-15-/-
D
CS Specific Activity (nmol/min/mg protein)
800
600
400
200
0
**
*
IL-15tg
B6
IL-15-/-

**Supplemental Figure 1**



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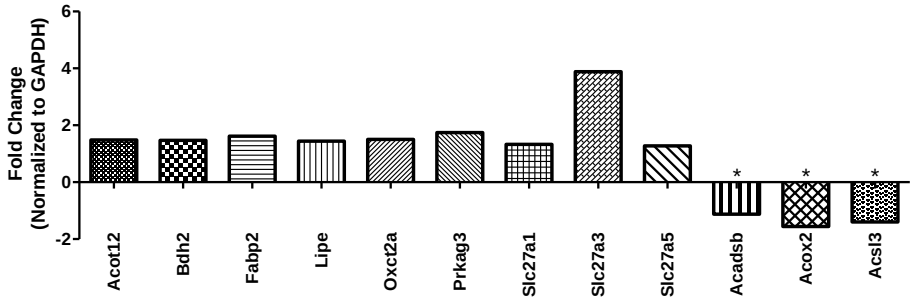

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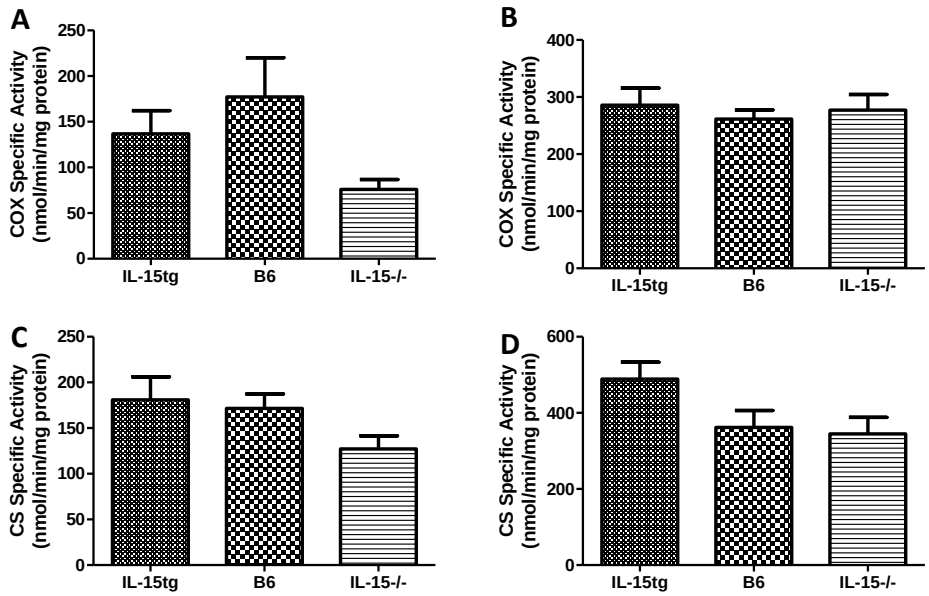
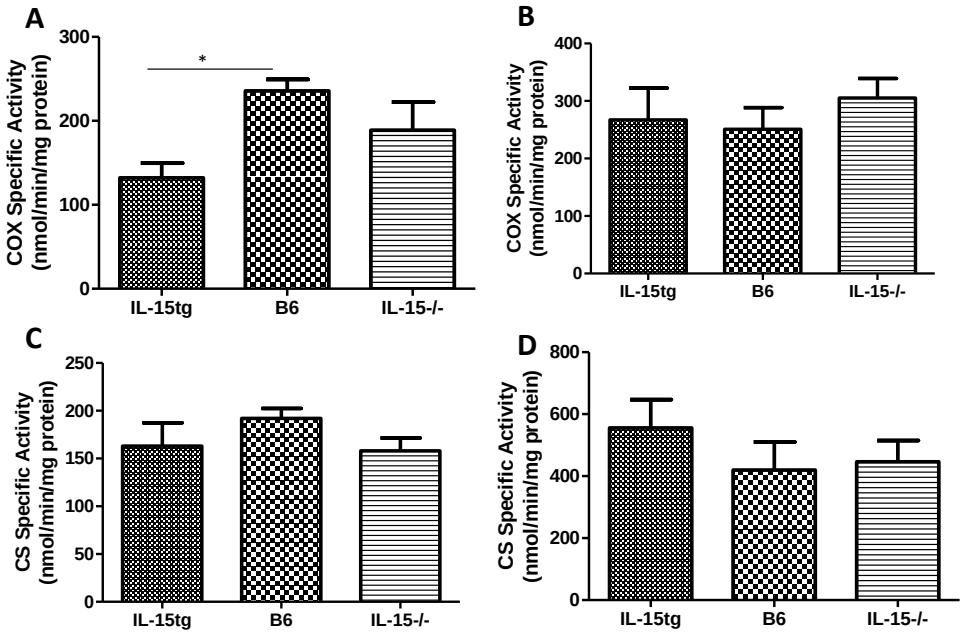


**Supplemental Figure 4**



# CHAPTER 5

# DISCUSSION

Cytokines involved in modulating immune responses are also associated with obesity and adiposity. The purpose of this thesis was to provide additional insights into IL-15's effects on adipose tissue regulation. Here, we show that low IL-15 concentrations are linked to increased body weight and adiposity in mice and humans (chapter 2) and that IL-15 mediated weight loss occurs independently of lymphocytes (chapter 3). Lastly, *in vitro* studies demonstrate that IL-15 may mediate its lipolytic effects directly, resulting in increased mitochondrial activity (chapter 4).

**IL-15 Expression Affects Body Weight and Adiposity**

Since BMI values are calculated based on body weight, therapeutic strategies against obesity have utilized methods aimed at initially decreasing and subsequently maintaining a 'healthy' body weight in obese patients. This decrease in weight loss targets a specific decline in adiposity, as obesity is defined as a state of abnormal adipose accumulation (WHO, 2013). We and a group from Copenhagen showed that circulating IL-15 is significantly lower in obese individuals compared to lean counterparts, and is negatively correlated to trunk fat mass, total fat mass, and percentage of fat mass (chapter 2). These negative correlations occurred independent of the subject's type 2 diabetic status (Barra et al., 2009; Nielsen et al., 2008). This demonstrates that low IL-15 levels are associated with obesity, and warrants further investigation into establishing IL-15's possible link to adiposity.

Studies completed by our group and others have revealed that IL-15 affects body weight and adiposity in genetically modified mice, as well as in rodents administered IL-15 using recombinant peptides or adenoviral vectors. Initial reports using daily delivery of 100μg/kg recombinant IL-15 peptide subcutaneously for seven consecutive days in male Wistar rats resulted in decreased dorsal and epididymal white adipose tissue mass while maintaining or increasing skeletal muscle mass (Almendro et al., 2006; Almendro et al., 2008; Carbo et al., 2001). Surprisingly, this treatment regime did not affect body weight despite significantly reducing white adipose tissue. Unlike this data, we showed that IL-15-/- mice given 500ng rmIL-15 intraperitoneally for seven consecutive days resulted in significant weight loss (chapter 2). Differences in the routes of cytokine administration and commercial sources of peptide may play a role in the differential effects of IL-15 on body weight observed in these recombinant protein treatments. Despite these discrepancies, our data corresponds to the finding that IL-15 administration results in a significant reduction in adiposity without decreasing skeletal muscle mass. As well, to further demonstrate IL-15's ability to decrease adiposity, similar results were found in relevant rodent models of obesity in IL-15 treated diet induced obese mice (chapter 2) and leptin deficient ob/ob mice (Alvarez et al., 2002).

We found that varying IL-15 expression also resulted in variation in body weight using IL-15 deficient and transgenic mice. Similar to our published results in obese individuals, we are the first group to show that genetically deficient IL-15-/- mice were

heavier and had increased visceral adiposity compared to control B6 and IL-15tg mice (chapter 2). Since then, studies have examined the metabolic effects of IL-15 overexpression on adiposity and body weight. Similar to our study, IL-15 overexpression was shown to result in decreased adiposity and body weight in mice. In a muscle specific transgenic mouse model, IL-15tg mice had decreased body weight and adiposity compared to control B6 mice when placed on either a low or high fat diet (Quinn et al., 2009). Transfected mice injected intramuscularly with plasmids expressing IL-15 also resulted in decreased trunk adiposity in mice when placed on either a standard diet or a 60% high fat diet compared to control counterparts (Nielsen et al., 2008). Lastly, it is important to note that IL-15 treatment, and IL-15 deficiency and overexpression did not affect food intake when compared to control animals in our studies (chapter 2-4) and in any previous studies (Almendro et al., 2006; Almendro et al., 2008; Carbo et al., 2001; Quinn et al., 2009). This suggests that IL-15's effects on body weight and adiposity occur independently of altered neuronal regulation of appetite. Altogether, evidence included in this thesis and provided by others demonstrate that IL-15 may regulate adipose tissue mass.

**IL-15 mediates differences in body weight independent of lymphocytes and the gut microbiota**

Considering IL-15 exerts various effects on immune cells found in adipose tissue as discussed in chapter 1, we examined the role of lymphocytes in mediating IL-15

induced weight loss in chapter 3. The effects of weight loss on adipose tissue have been investigated; however, the role(s) of innate and adaptive immunity during this process is not well described. Since studies have predominantly examined the role of immune cells in chronic metabolic disease development in obesity (discussed in chapter 1), further research is critical to determine whether immune cells play a role in mediating weight loss. Since IL-15 is primarily known for its effects on lymphocytes, we chose to examine this population and determined that IL-15 mediated weight loss occurs independently of this population.

Interestingly, research has recently demonstrated that immune myeloid derived macrophages may play a role during weight loss, suggesting immune cells may contribute to this metabolic process. Adipocyte size is strongly, positively correlated with ATM content (Weisberg et al., 2003), where sustained weight loss results in reduced macrophage number (Cancello et al., 2005). Upon further investigation, Kosteli and colleagues demonstrated that weight loss resulted in rapid, transient, recruitment of macrophages to adipose tissue followed by a progressive decline with continuous weight loss. Although macrophage polarization state was unaffected during this process, the researchers showed that liberated FFAs may act as a chemotactic factor for ATMS, and result in lipid uptake upon arrival. (Kosteli et al., 2010). Another study published by Nguygen and colleagues showed that M2 polarized ATMs secrete catecholamines, inducing thermogenic gene expression in brown fat and lipolysis in white fat in response

to a 4°C cold exposure (Nguyen et al., 2011). Aside from demonstrating that macrophages play a role in mediating responses to cold temperatures, this exciting data shows that immune cells have the ability to mediate lipolysis in white adipose tissue directly through the release of noradrenaline and adrenaline. Both studies illustrate that immune cells have the ability to influence metabolic processes such as weight loss, and cytokines like IL-15 may mediate its metabolic effects through immune-mediated mechanisms. While IL-15 induces weight loss in lymphocyte deficient RAG2-/-$\gamma_c$-/- mice, it is yet to be determined whether IL-15 may partially mediate its effects through myeloid derived immune cells.

The gut microbiota is another important contributing factor that has been recently examined for its involvement in differential weight gain and adiposity. As described in chapter 1, the gut microbiota refers to all the microorganisms inhabiting the intestine and is composed of predominately bacterial species. Changes in its composition correlate to a change in obese status and are thought to play significant roles in metabolic disease development and mediate differences in body composition. Possible mechanisms exerted by the gut microbiota include altering fermentation and subsequent absorption of macronutrients, and modulating genetic expression of factors involved in host metabolism (described in chapter 1). In chapter 4, we found significant differences among the intestinal bacterial profiles of IL-15tg, B6, and IL-15-/- mice; however, these differences did not translate to changes in intestinal macronutrient

absorption and body weight. Genetic deficiencies in immune factors like TLR-5 and lymophotoxin described in chapter 1 correlate to metabolic abnormalities and altered adiposity that were transferrable to germ free animals via colonization of respective gut bacterial colonies when compared to colonized control animals (Upadhyay et al., 2012; Vijay-Kumar et al., 2010). Despite these novel findings, much of our current knowledge aimed at uncovering the underlining mechanisms of the gut microbiota in obesity development is based on colonization experiments using germ free animals. Therefore, understanding the effect of gut microbial composition, rather than the effect of its absence or presence, is critical in determining its contribution to obesity and validity as a possible therapeutic strategy.

**IL-15 Directly Affects Adipose Tissue and Adipocytes**

To determine the underlining mechanisms by which IL-15 affects adiposity, several studies have examined whether IL-15 directly affects adipose tissue. Using cultured adipocytes *in vitro*, we (chapters 2 and 4) and others have shown that IL-15 treatment can directly affect mature adipocytes as well as cells at earlier stages of adipocyte development by decreasing lipid deposition. This demonstrates that IL-15 may play a significant role in different stages of adipocyte development. Similarly, we and others have shown that IL-15 treatment in culture significantly alters the expression of various adipokines like KC, adiponectin, and IL-6. Since the obese state is characterized by adipocyte hypertrophy and increased expression of proinflammatory adipokines

(described in chapter 1), understanding how IL-15 mechanistically affects the expression of adipokines and modulates adipocyte size may provide the basis for therapeutic strategies aimed at alleviating the proinflammatory environment defined in this disease state. Aside from the immunological effects IL-15 exerts on various immune cells, the capability of IL-15 to modulate adipocyte size may also affect the inflammatory status in adipose tissue by diminishing ATM content in adipose tissue (Weisberg et al., 2003).

A previous study has demonstrated that adipocytes express IL-15's heterotrimeric signaling complex at the mRNA level, further suggesting that IL-15 may induce its effects directly on adipocytes (Alvarez et al., 2002); however, whether fat cells express these receptor subunits on their cell surface and the mechanism(s) that mediate its effects are unknown. Our current knowledge of IL-15 mediated signaling has primarily focused on lymphocyte populations, where *trans*-signaling (described in chapter 1) has been well characterized. In chapters 3 and 4, we showed that IL-15 may mediate its effects on adipose tissue in the absence of the receptor-$\gamma_c$ subunit. Using RAG2-/-$\gamma_c$-/- mice, we demonstrated that IL-15 administration can induce weight loss, reduce adipocyte sizes, and upregulate markers of FAO, such as CPT-1α and CHAD, in adipose tissue. Previous reports from our laboratory have also demonstrated that IL-15 may mediate its effects in the absence of the $\gamma_c$. *In vivo*, delivery of IL-15 protected RAG2-/-$\gamma_c$-/- mice against a lethal vaginal herpes simplex virus (HSV)-2 challenge (Gill, Rosenthal, & Ashkar, 2005) and against lung metastasis after an injection with B16/F10 melanoma

cells (Davies et al., 2008). As well, IL-15 induced the production of the chemokine CLL5, also known as RANTES, in bone marrow derived myeloid cells taken from RAG2-/-$\gamma_c$-/- mice. Further analysis revealed that RANTES production occurred independently of IL-2/IL-15 β-receptor subunit, and required the IL-15 specific receptor-α subunit (Chenoweth et al., 2012). Altogether, these studies suggest that IL-15 can mediate its effects in the absence of the $\gamma_c$ and that IL-15Rα may be solely required. Further investigations to determine the contribution of each receptor subunit are essential to uncovering how IL-15 mechanistically affects adipocytes.

Lastly, previous studies and data presented in this thesis demonstrate IL-15 may promote lipolysis and FAO. In chapter 4, we showed that cultured adipocytes treated with recombinant IL-15 protein had decreased lipid deposition, suggesting that IL-15 administration promotes lipolysis. Lipolytic processes will favorably affect adipocyte size in the context of obesity, resulting in smaller fat cells; however, the effects of released NEFAs and glycerol due to lipolysis *in vivo* may not be beneficial and result in ectopic lipid deposition at other metabolic sites if not utilized. Ectopic lipid deposition is highly correlated with insulin resistance and therefore promotes metabolic disease development associated with obesity. Interestingly, treatment of mice with recombinant IL-15 promoted whole body and skeletal muscle FAO, as well as decreased lipid content in skeletal muscle (Almendro et al., 2006). As well, we showed that IL-15 administration resulted in the genetic upregulation of FAO markers in adipose tissue, such as CPT-1α

and CHAD. Along with our data presented in chapter 4, this suggests that IL-15 administration may result in lipolysis, promoting FAO locally in adipose tissue and at distant sites leading to decreased ectopic lipid content in tissues such as skeletal muscle. Since obesity is correlated to impairments in the FAO process, understanding how IL-15 promotes the mitochondrial utilization of fatty acids may alleviate ectopic lipid deposition found in obese subjects and improve insulin sensitivity. Whether these effects are seen in other insulin sensitive tissues, like the liver and cardiac muscle, and in murine obesity models have yet to be determined.

Our preliminary results presented in chapter 4 demonstrate that IL-15 induced significant mitochondrial effects within white adipose tissue by directly increasing its membrane potential in cultured adipocytes. Significant increase in the mitochondrial activity and mass specific to adipose tissue was also found in our IL-15tg mouse model. Mitochondrial dysfunction in obese adipose tissue is characterized by decreased mitochondrial DNA, mitochondrial mass, and mitochondrial activity (Choo et al., 2006; Rong et al., 2007). Our data suggests that in the presence of IL-15, mitochondrial mass and activity are increased in adipose tissue. Whether this increase in mitochondrial mass and activity is caused by an increase in mitochondrial number has yet to be determined. It would also be of interest to determine whether the IL-15 mediated mitochondrial changes affect other functional processes of mitochondria such as OXPHOS, ROS production, and the generation of ATP. A thorough understanding of how IL-15 affects

mitochondrial function(s) in adipose tissue is critical to determine its therapeutic potential. Further characterization of the effects of varying IL-15 expression on the mitochondria and its metabolic consequences is essential to fully elucidate the importance of altered mitochondria in promoting the phenotype of our three murine strains. However, since obesity is characterized as a state of mitochondrial dysfunction, these preliminary results suggest that IL-15 may alleviate obese mitochondrial dysfunction by promoting FAO within adipose tissue, as well as at other metabolic tissue sites.

**Clinical Relevance and Concluding Remarks**

Cytokines involved in modulating immune responses are also associated with obesity and adiposity. The primary purpose of this thesis was to provide additional insights into IL-15's effects on adipose tissue regulation. First, we showed that over time, over expression of IL-15 in mice was associated with a lean body condition, while IL-15 deficient mice gained significantly more weight, developing an obese phenotype when compared to control C57Bl/6 (B6) mice. We furthered these observational studies to demonstrate that acute IL-15 administration resulted in weight loss in IL-15-/- mice and in a relevant murine model of diet induced obesity. Furthermore, we found that circulating levels of IL-15 were significantly decreased in human obese individuals compared to lean subjects (chapter 2). Secondly, since IL-15 is known to generally mediate its effects through lymphocyte activation, we found that IL-15 treatment

induced weight loss in B6 and RAG2-/-$\gamma_c$-/- mice independent of lymphocytes (chapter 3). Thirdly, in an attempt to determine the underlying mechanism(s) mediating differences in body weight between our three strains of mice, we found differences in body weight occurred independently of food intake, activity, and macronutrient absorption. IL-15tg mice had increased mitochondrial activity and mass specific to adipose tissue, compared to IL-15-/- and B6 mice and acute IL-15 administration induced the expression of adipose tissue markers associated with FAO. Lastly, IL-15 can directly affect adipocytes by promoting increased mitochondrial membrane potential and decreased lipid deposition (chapter 4). Altogether, these results provide evidence that IL-15 may affect body weight and host adiposity through direct interactions with adipocytes and may provide insights into novel therapeutic strategies against obesity.

Further characterization of IL-15 mediated effects on adipose tissue is critical to uncovering IL-15's clinical relevance and therapeutic contribution to obesity. Current studies, including those described here, suggest that IL-15 may decrease adiposity by inducing FAO. If IL-15 promotes the oxidative process, this would tremendously benefit obese individuals who are insulin resistant due to increased adiposity and ectopic lipid deposition. Therefore, targeting factors like IL-15 that may promote mitochondrial FAO locally in adipose tissue and at distant insulin sensitive tissue sites may have substantial therapeutic benefit. Therapeutic strategies aimed at modulating IL-15 expression must not rely on chronic cytokine administration with such a broad-acting, pro-inflammatory

interleukin. Short term studies have demonstrated that IL-15 administration promotes decreased adiposity in various rodent models over seven to eight day time frames. Whether the effect of IL-15 on adiposity is sustained once cytokine administration is discontinued has yet to be determined. Although IL-15tg mice remain lean over a significant period of time (6 months – chapter 2), these mice may succumb to alopecia, skin lesions, and leukemia (Fehniger, Suzuki, Ponnappan et al., 2001; Fehniger, Suzuki, VanDeusen et al., 2001). These negative outcomes demonstrate possible hazardous effects of chronic, high level IL-15 treatment in obese subjects.

With its highest mRNA expression found in skeletal muscle, it has been suggested that IL-15 expression could be naturally induced through contracting muscle during exercise. Following a bout of strength training, IL-15 mRNA levels were upregulated in skeletal muscle of healthy male individuals (Nielsen et al., 2007). Treadmill running increased circulating IL-15 in untrained healthy male subjects and in diet induced obese rats compared to respective controls (Tamura et al., 2011; Yang et al., 2013). Further characterization of the type(s) and duration of exercise regimes and targeted muscle groups are needed to understand how exercise promotes optimal IL-15 expression and secretion. Also, it will be important to examine how exercise induced IL-15 expression contributes to body weight and host adiposity. In conclusion, the findings presented in this thesis broaden our understanding of the function of cytokines like IL-15 to include both its immune and metabolic effects. These findings suggest that further unraveling of

the mechanisms of IL-15 action may provide critical insights into adipose tissue biology and the basis for therapeutic strategies for the prevention of and protection against obesity.

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