# Influence of Anatomic Depot on the Apoptotic Susceptibility of Adipose Progenitor Cells

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**Abstract**

Adipose tissue (AT) expands through hypertrophy and hyperplasia. Hyperplasic AT expansion requires an adequate number of adipose progenitor cells. This study investigates the influence of depot origin on the susceptibility of adipose progenitors to cell death, and measures the effect of macrophage-secreted factors on adipose progenitor survival. Using serum deprivation alone or in the presence of TNFα, omental (OM) versus subcutaneous (SC) adipose progenitors, obtained from human AT, displayed a 3- and 1.7-fold-increase in apoptosis, respectively, as assessed by Hoechst staining, ($p<0.05$). Similar results were observed with cell enumeration. The ratio of OM/SC cell death from serum deprivation positively correlated with body mass index (BMI). The depot-specific difference in cell death was lost when TNFα and cycloheximide (CHX) were used. Monocyte-derived macrophages (MD-macrophages), isolated from human blood, did not have an effect on apoptosis. Depot-related differences in adipose progenitor apoptosis may influence AT remodeling and alter metabolic functionality in obesity.

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## List of Abbreviations

| | |
|---|---|
| ANOVA | Analysis of variance |
| AT | Adipose tissue |
| ATM | Adipose tissue macrophage |
| ATP | Adenosine triphosphate |
| BAT | Brown adipose tissue |
| BMI | Body Mass Index |
| C/EBP | CCAAT-enhancer binding protein |
| CAD | Caspase-activated DNase |
| CD | Cluster of differentiation |
| CHX | Cycloheximide |
| cIAP | Cellular Inhibitor of Apoptosis |
| CLS | Crown-like structure |
| CT | Computed tomography |
| CVD | Cardiovascular disease |
| DEXA | Dual-energy X-ray absorptiometry |
| DISC | Death inducing signaling complex |
| DMEM | Dulbecco's modified eagle's medium |
| EDTA | Ethylenediaminetetraacetic acid |
| ER | Endoplasmic reticulum |
| FADD | Fas-Associated Protein with Death Domain |
| FBS | Fetal bovine serum |
| FFA | Free fatty acids |
| FLICE | Fas-associated protein with death domain-like interleukin-1 beta-converting enzyme |
| FLIP | FLICE-like inhibitory protein |
| FTO | Fat mass and obesity associated |
| GLUT4 | Glucose transporter type 4 |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| GWAS | Genome-wide association studies |
| HIF-1α | Hypoxia-inducible factor-1 alpha |
| IAP | Inhibitor of apoptosis |
| ICAD | Inhibitor of caspase-activated DNase |
| IGF | Insulin growth factor |
| IKKβ | Inhibitor of nuclear factor kapp B kinase subunit beta |
| IL | Interleukin |
| IRS-1 | Insulin receptor substrate-1 |
| JNK | c-Jun N-terminal kinase |
| LD | Lipid droplet |
| LPS | Lipopolysaccharide |
| MacCM | Macrophage-conditioned medium |
| MCP-1 | Monocyte chemotactic protein-1 |
| MD-macrophages | Monocyte-derived macrophages |
| MHO | Metabolically healthy obese |
| MRI | Magnetic resonance imaging |
| NAFLD | Non-alcoholic fatty liver disease |

| | |
|---|---|
| NAIP | NLR apoptosis inhibitory protein |
| NEFA | Non-esterified fatty acids |
| NFκB | Nuclear factor kappa B |
| OAT | Omental adipose tissue |
| OM | Omental |
| PBMC | Peripheral blood mononuclear cell |
| PBS | Phosphate buffered saline |
| PDGF | Platelet-derived growth factor |
| PPARγ | Peroxisome proliferator-activated receptor gamma |
| RIPK3 | Receptor-interacting protein kinase 3 |
| ROS | Reactive oxygen species |
| RPMI | Roswell Park Memorial Institute |
| SAT | Subcuatenous adipose tissue |
| SC | Subcutaneous |
| SGBS | Simpson-Golabi-Behmel syndrome |
| SVF | Stromal vascular fraction |
| T2D | Type 2 diabetes |
| TG | Triacylglycerol |
| $T_H1$ | Type 1 helper T cells |
| $T_H2$ | Type 2 helper T cells |
| TNFα | Tumor necrosis factor alpha |
| TNFR | Tumor necrosis factor receptor |
| TRAIL | TNF-related apoptosis-inducing ligand |
| Tregs | T regulatory cells |
| UPR | Unfolded protein response |
| VAT | Visceral adipose tissue |
| WAT | White adipose tissue |
| WC | Waist circumference |
| WT | Wild type |
| zVAD-FMK | Carbobenzoxy-valyl-alanly-aspartyl-(β-*O*-methyl)-fluoromethyl ketone |

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## 1.0 General Introduction

## Obesity

### *Definition and Assessment*

Obesity is defined as an excess accumulation of fat mass (adipose tissue; AT). Since the 1970's, the prevalence of obesity has increased significantly worldwide. Estimates from 2009 indicate that ~24% of Canadians are obese, representing a large increase from ~14% in 1989 (McGuire et al., 2011). Increases in the prevalence of obesity have been substantial, widespread, and have arisen over a short time. However, there is some evidence of a plateau in adult and childhood obesity rates that provides some hope that the epidemic might have peaked in some developed countries (Ng et al., 2014).

There are numerous methods to measure obesity, with advantages and disadvantages to each. Measurements of body weight and height are used most frequently as they provide a rapid and economical way to determine adiposity. Body mass index (BMI) is calculated as weight in kilograms (kg) divided by height in meters squared ($m^2$). Individuals with a BMI $\geq$ 25 kg/$m^2$ are considered overweight and those with a BMI $\geq$ 30 kg/$m^2$ are classified as obese. Because the risk of adverse health effects increases at higher levels of BMI, obesity is divided into three categories. For male and female adults aged 20 and over, obesity class I is defined as a BMI of 30.0 to 34.9 kg/$m^2$ obesity class II is defined as a BMI of 35.0 to 39.9 kg/$m^2$ and obesity class III is defined as a BMI of 40.0 kg/$m^2$ or higher. BMI has some limitations in the analysis of fat mass. The calculation is solely dependent on the net weight and height of the individual and does not consider the distribution of muscle and bone mass.

There is a potential risk of overestimating adiposity in individuals with high muscle mass and underestimating the fat deposit in those with less lean body mass.

In contrast to BMI, waist circumference (WC) is a better indicator of central adiposity (Knowles et al., 2011). It is an indicator of health risk associated with excess fat around the waist (also described as an 'apple' body shape). A WC of 102 cm (40 inches) or more in men, or 88 cm (35 inches) or more in women, is associated with health problems such as type 2 diabetes (T2D), cardiovascular disease (CVD) and hypertension. Even if the BMI of an individual is in the normal weight range, a high WC demonstrates some health risk.

Historically in the research setting, total body fat was measured by densitometry based on the principles of water displacement, assuming just two body compartments: fat (density about 0.7 g/ml) and fat-free tissue (about 1.0 g/ml) (Han et al., 2006). According to this principle, if two individuals of the same weight on land have different proportions of body fat and lean tissue, the one with more body fat and less lean tissue would weigh less under water. Today, densitometry is not frequently used as it is labour-intensive, expensive and fails to indicate body fat distribution.

Imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI) and dual-energy X-ray absorptiometry (DEXA) allow whole body or regional assessments of body fat. CT and MRI are considered to be the most accurate methods for measuring tissue, organ and whole-body fat mass. Specific abdominal AT depots can be measured, including the subcutaneous (SC) and visceral depots. DEXA quantifies bone mineral density and lean soft tissue, from which AT mass is indirectly estimated (Manninen, 2006). DEXA cannot accurately distinguish between different types of fat. Imaging is very

expensive and some techniques (CT and DEXA) can expose patients to radiation, so its use is limited, especially with repeated measurements (Han et al., 2006).

### *Causes of Obesity*

AT mass depends on the relative intake versus expenditure of calories. In a state of chronic positive energy imbalance, AT expands and obesity ensues. Hypernutrition and a sedentary lifestyle are the main factors contributing to obesity; however, genetic predisposition also has a profound effect on energy balance. Genetic influences on obesity have been estimated to contribute 40-70% to the variation in body weight within a population, by affecting energy intake and expenditure (Tung and Yeo, 2011). Although obesity is generally considered a multifactorial disease, monogenic forms of obesity exist. Monogenic forms of obesity usually result in an early-onset severe form of weight gain. Melanocortin 4 receptor loss-of-function mutations represent the most common monogenic form of obesity, accounting for ~5% of severe early onset obesity (Calton et al., 2009; Hainerova et al., 2007; Hinney et al., 2006; Krakoff et al., 2008; Lubrano-Berthelier et al., 2006; Stutzmann et al., 2008). This hypothalamic receptor recognizes α-melanocyte stimulating hormone and signals to decrease food intake. Loss-of-function mutations in the leptin gene or the leptin receptor account for ~3% of severe cases of obesity (Farooqi et al., 2002). Leptin was the first weight-regulating adipocyte secreted factor (adipokine) to be identified (Zhang et al., 1994; Halaas et al., 1994). Leptin exerts pleiotropic effects on metabolism and energy homeostasis by acting on both central and peripheral tissues (Bjorbaek and Kahn, 2004; Lago et al., 2007) and reduces appetite by acting on its hypothalamic receptor (Montague et al., 1997).

It is believed that the remaining heritability lies within a large number of genetic variants affecting multiple genes (Ramachandrappa and Farooqi, 2011). In order to identify genetic mutations that associate with obesity, many genome-wide association studies (GWAS) have been conducted. GWAS have currently identified ~30 genetic loci affecting the risk of developing obesity (McCarthy, 2010). Currently, loci identified by GWAS account for ~10% of the heritability of obesity (Ramachandrappa and Farooqi, 2011). The first and strongest identified association between body weight and multiple variants in genetic loci was the fat mass and obesity associated FTO gene. Mutations in the FTO loci are associated with a 3 kg increase in body weight and a 1.7-fold increased risk of obesity (Frayling et al., 2007). A recent study (Smemo et al., 2014) revealed that the obesity-associated noncoding sequences within *FTO* are connected with the homeobox gene *IRX3*. The obesity-associated *FTO* region directly interacts with the promoters of *IRX3* as well as *FTO* in human, mouse and zebrafish. Furthermore, obesity-associated single nucleotide polymorphisms are associated with expression of *IRX3*, but not *FTO*, in human brains. A reduction in body weight of 25 to 30% was observed in *Irx3*-deficient mice, through the loss of fat mass and increase in basal metabolic rate. *IRX3* is a target of obesity-associated variants within *FTO* and may provide a new determinant of body mass and composition.

### *Risks of Obesity*

Obesity is a growing public health concern throughout the world because it is associated with many diseases. As previously mentioned, it is caused by a combination of environmental and genetic factors. Obesity is associated with cardiac and metabolic risk factors which lead to increased morbidity and mortality. Cardiometabolic complications are related to a broad spectrum of pathological conditions, including insulin resistance, T2D,

non-alcoholic fatty liver disease (NAFLD), dyslipidemia, hypertension, CVD and some types of cancer (Conway and Rene, 2004; Khandekar et al., 2011).

It is widely believed that insulin resistance is the main etiological factor that contributes to the development of obesity-related complications. Even though obesity is commonly linked to the onset of cardiometabolic abnormalities, not all obese individuals suffer from metabolic dysfunction and insulin resistance. This subset of apparently healthy obese subjects is referred to as metabolically healthy obese (MHO). Several studies have confirmed the existence of MHO individuals, accounting for as much as 25% of the obese population (Karelis, 2008; Karelis et al., 2004; Messier et al., 2010; Primeau et al., 2011). MHO individuals are characterized by normal insulin sensitivity, a low prevalence of hypertension, and a favourable lipid and inflammation profile (Hinnouho et al., 2013).

Nutrient excess causes AT to undergo rapid and dynamic changes through adipocyte hypertrophy (increase in adipocyte size) and hyperplasia (increase in adipocyte number). In an obese state, AT remodeling is often associated with pathological changes. However the idea of a MHO state suggests that some individuals avoid the pathological consequences associated with obesity on the basis of healthy AT expansion primarily by hyperplasia. For this reason it is crucial to consider mechanisms of AT expansion.

## Adipose Tissue

### *Cellular Composition of Adipose Tissue*

Adipocytes constitute the main cellular component of white AT (WAT) and comprise between 50-70% of the cellular mass of AT (Hauner, 2005). In addition to adipocytes, WAT contains non-adipocyte cellular components which are collectively referred to as the stromal vascular fraction (SVF). These cells include adipose progenitor cells (preadipocytes) (20-

40%), fibroblasts, smooth vascular endothelial cells (1-10%) and a variety of immune cells (10-50%), including AT macrophages (ATMs).

### *Adipose Progenitor Cells*

AT is derived from the mesenchyme and contains stromal progenitors at various stages of adipogenic commitment. Less committed progenitors (mesenchymal stem cells) can differentiate into several cell types, including osteoblasts, chrondrocytes, myoblasts and adipocytes when provided with the appropriate signals. Adipose progenitors are defined as either adherent stromal cells possessing adipogenic potential, or as stromal cells expressing a limited number of cell surface proteins (Rodeheffer et al., 2008; Tchoukalova et al., 2007; Zuk et al., 2002; Tran et al., 2012; Cawthorn et al., 2012). Methods for human adipose progenitor cell identification vary due to a lack of consistent cell surface markers capable of distinguishing multipotent cells from committed adipose progenitor cells, in contrast to more defined protocols for murine cells.

Committed adipose progenitor cells undergo growth arrest and subsequent terminal differentiation into adipocytes (Tang and Lane, 2012). Exposure of growth-arrested adipose progenitors to differentiation inducers (IGF1/insulin, glucocorticoid and cAMP-elevating agents) triggers DNA replication, re-entry into the cell cycle (a process known as mitotic clonal expansion) and an adipogenic transcription factor cascade. These events lead to expression of adipocyte genes. Peroxisome proliferator-activated receptor gamma (PPARγ) is the master regulator of adipogenesis. PPARγ is a member of the nuclear-receptor superfamily, and is both necessary and sufficient for adipogenesis. Several CCAAT-enhancer binding proteins (C/EBPs) participate in adipogenesis. Early induction of C/EBPβ and C/EBPδ during adipogenesis leads to the up regulation of C/EBPα during the terminal

stages. As a result, the transcription of lipogenic genes is activated, including those that support fatty acid synthesis, desaturation, and uptake, as well as triacylglycerol (TG) synthesis.

### *Adipocytes*

The adipocyte serves as the energy reservoir within AT. Adipocytes contain a single lipid droplet (LD), consisting of a phospholipid monolayer encapsulating a neutral lipid core. The lipid core is composed primarily of TG (Frayn et al., 2003). TG is synthesized when energy is in excess. Lipolysis within the adipocyte LD occurs to meet energy demand and is defined as the breakdown of TG into glycerol and individual non-esterified fatty acids (NEFA). Furthermore, adipocytes are unique in that they do not form a lot of cholesterol ester.

Cellular dating analysis has shed new light on the turnover of adipocytes. A comparison of $^{14}C$ levels in adipocyte genomic DNA with declining atmospheric $^{14}C$ levels estimated that ~10% of adipocytes within human SC tissue turn over each year (Spalding et al., 2008) . The number of fat cells stays constant in adulthood in lean and obese individuals, even after marked weight loss, indicating that the number of adipocytes is set during childhood and adolescence (Spalding et al., 2008). The notion that the total number of fat cells remains constant in adults has been challenged (Tchoukalova et al., 2010). Since adipocytes are post-mitotic in nature, generation of new adipocytes occurs via the recruitment and differentiation of adipogenic progenitor cells.

### *Forms and Function*

AT exists in two forms, WAT and brown AT (BAT), each possessing unique functions. WAT stores excess energy as triglycerides and BAT is specialized in the dissipation of energy through the production of heat.

BAT is a thermogenic organ producing heat by uncoupling mitochondrial electron transport from adenosine triphosphate (ATP) production. This is made possible by the expression of uncoupling protein-1 (UCP-1) in the mitochondria of brown adipocytes (Cannon et al., 1982). Although human BAT was long thought to disappear after birth, recent positron emission tomography imaging studies have revealed functional BAT in adults, located primarily in the neck region (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009) . Uncoupled oxidation of NEFA within brown adipocytes significantly contributes to whole body energy expenditure during cold exposure (Ouellet et al., 2012).

WAT is a highly active endocrine organ that is critical for the regulation of energy storage within the body during periods of fasting and feeding. In the fed state, insulin increases energy uptake and storage in AT (glucose uptake and lipogenesis) and decreases energy breakdown and release from AT (lipolysis) (Frayn et al., 2003). In the fasting state, and during periods of exercise, sympathetic innervation releases norepinephrine within AT to stimulate lipolysis (Frayn et al., 2003).

The major white and brown adipose depots are located in anatomically different regions in both rodents and humans. Beige AT is a recently identified AT composed of UCP-1 positive adipocytes and is present in scattered deposits in adult human WAT (Harms and Seale, 2013). Beige cells can activate a thermogenic mechanism that burns stored fat to

produce heat. Even though beige and brown fat share similar characteristics, they arise from different cellular lineages.

In the interest of our laboratory, my studies focus on WAT.

### *Endocrine Function of Adipose Tissue*

AT is an endocrine organ that secretes numerous molecules that affect the function of other tissues (Lago et al., 2007). Signaling molecules released from AT are referred to as adipokines. Adipokines influence food intake, systemic insulin sensitivity, nutrient balance and play a role in muscle insulin sensitivity and glucose uptake, liver metabolism, and the vasculature (Lau et al., 2005; Mora and Pessin, 2002). Additionally, adipokines are important immune regulatory molecules, acting as either anti-inflammatory (adiponectin, Interleukin (IL)-10), or pro-inflammatory (IL-6, IL-1β, tumor necrosis factor alpha; TNFα) autocrine/paracrine signals (Bradley et al., 2008; Hotamisligil et al., 1995; Ouchi et al., 2010; Turer and Scherer, 2012). These molecules are produced from a variety of cell types within the AT, including adipocytes, adipose progenitor cells, and immune cells (Hotamisligil et al., 1993; Shoelson et al., 2006; Romeo et al., 2012; Hotamisligil and Erbay, 2008).

### *Adipose Tissue Depots*

AT is a multi-depot organ composed of SC fat (beneath the skin), and visceral fat (intra-abdominal fat), found around internal abdominal organs. AT depots differ with respect to location, structure and function. Lineage tracing experiments have suggested distinct progenitor subpopulations may give rise to adipocytes in different depots (Chau et al., 2014; Seale et al., 2011). Distinct adipocyte origins and local microenvironment may contribute to the differences in metabolic activity of each depot.

The majority of SC fat is deposited in the femoral-gluteal regions, back and anterior abdominal wall. About 80% of all body fat is SC (Ibrahim, 2010). Abdominal fat is present in two main depots: SC and intra-abdominal. Visceral AT (VAT) is present around abdominal viscera in the mesentery and in the omentum. The greater omentum is the largest peritoneal fold within the abdomen and is known for its immunologic function. The omentum aids in isolating peritoneal infection and absorbing contaminants through the mesothelial stomata (Collins et al., 2009). Visceral fat accounts for up to 10-20% of total fat in men and 5-8% in women and tends to increase with age in both genders (Wajchenberg, 2000).

As compared to VAT, SC fat is more cellular, that is, it has a higher proportion of smaller cells because of the higher differentiation potential of subcutaneous adipocytes (Walker et al., 2007). SC fat is more densely vascularized than visceral fat, and it retains higher angiogenic potential (Gealekman et al., 2011).

Adipocyte progenitor cells isolated from VAT and SC AT (SAT) differ with respect to their ability to proliferate and differentiate into adipocytes (Macotela et al., 2012; Tchkonia et al., 2007). PPARγ-agonist-stimulated adipose progenitor cell differentiation is higher in SAT versus VAT (Walker et al., 2008).

Human visceral adipocytes have higher basal and catecholamine-induced lipolytic activity as compared to SAT (Ahmadian et al., 2010). Accordingly, the majority of circulating free fatty acids (FFA) is derived from the SC fat. GLUT4 and IRS-1 mRNA levels are lower in omental AT (OAT) than in SAT (Veilleux et al., 2009). As compared to SC fat cells, visceral adipocytes express less adiponectin (Yang et al., 2008) and adiponectin receptor (AdipoR1) (Rasmussen et al., 2006). Similarly, leptin mRNA expression and protein

secretion is lower in OAT (Van Harmelen et al., 1998). Generally, VAT secretes more pro-inflammatory cytokines, such as IL-6, than the SC fat depot (Fontana et al., 2007). Such an expression profile contributes to systemic inflammation associated with visceral obesity and obesity-associated metabolic disorders like insulin resistance and CVD (Torres-Leal et al., 2010; Fontana et al., 2007).

## Adipose Tissue Remodeling

### *Adipose Tissue Expansion*

Accumulation of AT occurs through hyperplasia and hypertrophy (Gesta et al., 2007). Differentiation of adipose progenitor cells into mature adipocytes is crucial to maintain normal function of AT and to meet energy storage demands.

Hyperplastic AT expansion involves the recruitment and differentiation of adipose progenitor cells into adipocytes. Expansion predominantly through hyperplasia distributes the excess energy among many newly generated adipocytes, which serves to maintain the metabolic function of each adipocyte. Hyperplastic AT expansion is associated with preserved insulin sensitivity in humans and animal models. An increase in adipocyte number depends on a sufficient number of functional adipose progenitor cells to form new adipocytes (Heilbronn et al., 2004).

Hypertrophic AT expansion occurs when excess energy is stored as triglycerides in existing adipocytes, resulting in hypertrophy (Heilbronn et al., 2004). During a state of positive caloric imbalance, a deficit in the number of adipocyte progenitor cells and/or their ability to differentiate or proliferate would impair hyperplastic AT growth (Strissel et al., 2007). As a result, AT expansion predominantly through hypertrophy will occur (Danforth,

2000; Heilbronn, et al., 2004). Several studies suggest an adipogenic deficit may exist in obese humans. The total number of adipose progenitor cells is reduced in obese individuals compared to lean individuals (Onate et al., 2012; Tchoukalova et al., 2007). Adipocyte hypertrophy is observed in AT sampled from obese patients, which correlates with an impaired adipogenic capacity of isolated adipose progenitor cells (Kloting et al., 2010; van Harmelen et al., 2003). Expression analysis has shown that adipogenic-related genes from AT sampled from insulin-resistant, obese individuals are downregulated relative to AT collected from MHO individuals (Kloting et al., 2010). Obese patients with hypertrophic AT and insulin resistance generate 70% fewer adipocytes per year compared to MHO patients with hyperplastic AT (Arner et al., 2010; Arner and Spalding, 2010). The number of adipose progenitor cells and their adipogenic capacity appears to influence AT function.

Weight gain through AT hyperplasia is commonly seen in MHO individuals, which represent up to 25% of obese humans (Bluher, 2010; Kloting et al., 2010). Therefore hypertrophic AT can be thought of as dysfunctional fat, whereas hyperplastic AT represents a functional form of fat.

### *Adipose Tissue Dysfunction*

The molecular mechanism by which adipocyte hypertrophy results in dysfunctional AT is complex. An excess of lipid within the adipocyte leads to endoplasmic reticulum (ER) stress and hypoxia, which consequently results in the production and secretion of pro-inflammatory cytokines.

### *Endoplasmic Reticulum Stress*

Excess lipid storage appears to cause functional abnormalities of the ER and mitochondria that are central to the pathophysiologic effects of obesity. The storage and handling of intracellular lipids requires the synthesis of many proteins necessary for fatty acid transport, modification, esterification, and packaging within lipid droplets (de Ferranti and Mozaffarian, 2008). The ER is an organelle that serves many functions, one of which includes the synthesis of nascent proteins by translating messenger RNA into a polypeptide sequence. When there is a continuous need to store TG, adipocytes must expand in size while continuously being stressed to synthesize more proteins for LD formation (Boden, 2009; Zha and Zhou, 2012). When the demand for protein synthesis exceeds the capacity of the ER, an unfolded protein response (UPR) is initiated. Under normal conditions, the UPR involves the activation of double-stranded RNA-dependent protein kinase-like ER kinase, inositol-requiring protein-1, and activating transcription factor 6, which act to restore ER function by upregulating protein chaperones, stimulating ER biogenesis, and inhibiting protein translation (Boden, 2009). Stress to the ER occurs when the increased demand for protein synthesis continues. Lipid accumulation in adipocytes causes the activation of JNK and IKKβ. These inflammatory signaling pathways regulate protein phosphorylation and transcriptional events leading to pro-inflammatory cytokine production (TNFα, IL-6, adiponectin, leptin, monocyte chemotactic protein-1; MCP-1) (Jiao et al., 2011; Hirosumi et al., 2002). ER stress is observed in adipose tissue of insulin-resistant obese mice and humans, and inhibition of this stress in mice improves insulin sensitivity (Boden, 2009; Ozcan et al., 2004; Ozcan et al., 2006).

### *Hypoxia*

Healthy expansion of AT depends on uniform vascularization of the growing tissue (Wronska and Kmiec, 2012). Inadequate vascularization results in an insufficient supply of $O_2$. In an obese state, as AT mass expands, clusters of adipocytes become hypoxic due to their distance from the tissue microvasculature. Hypoxia in AT has been linked to insulin resistance and the metabolic dysfunction (Trayhurn et al., 2008).

Under hypoxic conditions, hypertrophied adipocytes increase expression of the hypoxia-dependent protein known as hypoxia-inducible factor-1 alpha (HIF-1α) (Rausch et al., 2008). The primary function of HIF-1α is to promote vascularization through the upregulation of pro-angiogenic factors. Nevertheless, HIF-1α also signals through the pro-inflammatory transcription factor, nuclear factor kappa B (NFκB), turning on the expression of numerous insulin desensitizing pro-inflammatory genes, including TNFα, IL-1β, IL-6 and MCP-1. Adipocyte-specific deletion of HIF-1α appears to provide protection from obesity-associated insulin resistance (Jiang et al., 2011).

### *Adipose Tissue Inflammation*

Obesity-associated chronic AT inflammation is a key contributing factor to metabolic and cardiovascular dysfunction (Van Gaal et al., 2006). Numerous studies have shown that the immune system and metabolism are highly integrated (McNelis and Olefsky, 2014). A variety of immune cells reside in AT. The composition of AT immune cells includes myeloid cells from the innate immune system, such as macrophages and eosinophils, and lymphoid cells from the adaptive immune system, such as T and B lymphocytes (Makki et al., 2013). The immune cell population of AT is predominantly classified into two groups: pro-

inflammatory or anti-inflammatory (Makki et al., 2013). The balance between these populations seems to influence AT function and homeostasis.

In a non-obese state, AT $T_H2$ T cells, $T_{reg}$ cells, eosinophils and M2-like resident macrophages predominate. $T_{reg}$ cells and eosinophils and M2-like macrophages secrete cytokines that contribute to the anti-inflammatory, insulin-sensitive phenotype. In obesity-induced inflammation, immune cells are recruited from the circulation and contribute to AT inflammation. Monocytes respond to chemotactic signals and transmigrate into the AT and become polarized to the highly proinflammatory M1-like state (Osborn and Olefsky, 2012). Once recruited, these M1-like macrophages secrete proinflammatory cytokines that work in a paracrine fashion. Obesity causes the eosinophil content to decline and induces a shift in AT T cell populations with a decrease in $T_{reg}$ content and an increase in $CD4^+$ $T_H1$ and $CD8^+$ effector T cells, which secrete proinflammatory cytokines. B cell numbers also increase and activate T cells, which potentiate M1-like macrophage polarization, inflammation and insulin resistance. Cytokines and chemokines from the AT can also be released into the circulation and work in an endocrine manner to promote inflammation in other tissues (Osborn and Olefsky, 2012).

### *Adipose Tissue Eosinophils*

Eosinophils are associated with parasitic infections, allergies and asthma. In a lean state, AT eosinophils secrete IL-4 and IL-13 which contributes to the anti-inflammatory, insulin-sensitive phenotype. AT M2-like macrophages maintain glucose homeostasis and are induced by IL-4. One study demonstrated that eosinophils are the major IL-4-expressing cells in WAT of mice, and, in their absence, M2-like macrophages are greatly attenuated. Eosinophils migrate into AT by an integrin-dependent process and reconstitute M2-like

macrophages through an IL-4- or IL-13-dependent process. In an obese state, eosinophils appear to decrease in number.

### *Adipose Tissue T Cells*

AT contains innate immune cells which work in conjunction with adaptive immune cells. The adaptive immune system includes T and B cells, which respond to specific peptide antigens. T cells were first discovered to reside within AT in 2007 (Wu et al., 2007). T cells exist as both resident and recruited populations in AT. In a non-obese state, T regulatory cells (Tregs), which represent a subpopulation of T cells, diminish innate and adaptive immune reactions. They secrete IL-10 and stimulate IL-10 secretion from M2-like macrophages. Tregs represent ~ 3% of all white blood cells present in AT (Feuerer et al., 2009; Sun et al., 2012). The AT Treg population seems to decrease with the enlargement of adipose mass, and is associated with more AT inflammation (Feuerer et al., 2009; Nishimura et al., 2009).

### *Adipose Tissue B Cells*

B cells, defined by expression of CD19, are present in AT and increase in number as obesity develops (Duffaut et al., 2009). The increase in B cell population appears to activate T cells which sustain an M1-like population, and thus contributes to obesity-associated insulin resistance and AT inflammation (McDonnell et al., 2012; Osborn and Olefsky, 2012).

### *Adipose Tissue Macrophages*

ATMs may be associated with inflammation, or alternatively, tissue remodeling and resolution of inflammation, depending on the extent of adiposity and activation state of the macrophage. Macrophage-secreted paracrine factors may play a role in AT homeostasis,

including progenitor cell survival, proliferation and differentiation into adipocytes (Molgat et al., 2011; Molgat et al., 2012; Calder et al., 2011; Dalmas et al., 2011). Pro-inflammatory ATMs may promote hypertrophic AT expansion by inhibiting adipose progenitor cell proliferation and differentiation, whereas anti-inflammatory ATMs may promote hyperplastic and metabolically functional AT expansion through the maintenance of an adequate number of responsive progenitors (Molgat et al., 2012; Sorisky et al., 2013). Our laboratory has previously shown that macrophage-conditioned medium (MacCM) promotes 3T3-L1 adipose progenitor cell survival in a platelet-derived growth factor (PDGF) receptor-dependent manner. Furthermore, we showed that the MacCM pro-survival effect observed with the 3T3-L1 cell line model extends to interactions between human MD-macrophages and human subcutaneous adipose progenitor cells Molgat et al., 2012). However, the survival response to macrophage-secreted factors between different depots has not been investigated. Depot differences in apoptotic susceptibility between the two progenitor pools may provide insight into the pathogenesis of obesity and metabolic dysfunction.

**Cell Death**

There are two types of cell death: programmed and non-programmed. Programmed cell death is mediated by an intracellular process and is carried out in a controlled manner. Apoptosis and pyroptosis are forms of programmed cell death. Furthermore, necrosis is an example of a non-programmed process that occurs as a result of infection or injury. Recently, necroptosis has been recognized as an alternate form of programmed necrosis.

Apoptosis is the most characterized type of programmed cell death because of its importance in development, aging, homeostasis and in the pathogenesis of different diseases such as cancer. Apoptotic cells die in a controlled manner in response to a variety of extrinsic

(activation of TNF receptors) or intrinsic signals (DNA damage, mitochondrial disruption) (Igney and Krammer, 2002; Elmore, 2007). The hallmarks of apoptotic cell death include exposure of phosphatidylserine on the extracellular face of the plasma membrane, activation of caspases, disruption of mitochondrial membrane potential, cell shrinkage, DNA fragmentation and DNA condensation (Hacker, 2000; Kerr et al., 1972).

Pyroptosis is a cell death pathway that is caspase 1-dependent and inherently inflammatory (Fink and Cookson, 2005; Bergsbaken et al., 2009). Caspase-1 is not involved in apoptotic cell death and caspase-1-deficient cells respond normally to most apoptotic signals. Pyroptosis is triggered by pathological stimuli, such as stroke, heart attack or cancer, and is also crucial for controlling microbial infections (Fink and Cookson, 2005; Bergsbaken et al., 2009).

Necrosis is an uncontrolled cell death characterized by cell swelling, as well as destruction of the plasma membrane and subcellular organelles, without nuclear fragmentation and condensation (Linkermann and Green, 2014). Necrotic cell death is considered a heterogeneous phenomenon including both programmed and accidental cell death. Necrosis is often defined in a negative manner, as a type of cell death that involves rupture of the plasma membrane without the controlled mechanisms of apoptosis. Necrosis is considered to be a toxic process where the cell is a passive victim and follows an energy-independent mode of death.

Necroptosis, programmed necrosis, is a caspase-independent form of cell death that can be induced with TNFα only in the presence of a caspase inhibitor, such as zVAD-FMK (Linkermann and Green, 2014). It is dependent on receptor-interacting protein kinase 3 (RIPK3) and formation of the necrosome. Several upstream signaling elements of apoptosis and necroptosis overlap, and sensitivity to each death pathway is regulated (sometimes in

opposing ways) by shared regulatory molecules, such as FLICE-like inhibitory protein (FLIP) and the cellular inhibitors of apoptosis proteins cIAP1 and cIAP2.

The protein synthesis blocker cycloheximide (CHX) sensitizes human adipocytes for CD95-induced apoptosis in a caspase-dependent manner (Fischer-Posovszky et al., 2011). Treatment with CHX changes the expression of pro- and anti-apoptotic proteins. FLIP expression decreases during CHX treatment. Reduction of FLIP levels results in undetectable amounts of FLIP at the CD95 death-inducing signaling complex (DISC) upon CD95 stimulation, thereby enhancing recruitment and activation at caspase-8. Down-regulation of FLIP by shRNA sensitizes adipose progenitor cells for CD95-induced apoptosis. In mice, AT mRNA levels of Flip are down-regulated upon fasting. Therefore, FLIP is an important regulator of apoptosis sensitivity in fat cells.

## Apoptosis

### *Morphology*

Distinct morphological changes occur during apoptosis (Hacker, 2000). During the early process of apoptosis, cell shrinkage and pyknosis are visible by light microscopy (Kerr et al., 1972). The cytoplasm is dense and the organelles are more tightly packed. Pyknosis, the result of chromatin condensation, is the most characteristic feature of apoptosis. On histologic examination with hematoxylin and eosin stain, apoptosis involves single cells or small clusters of cells. The apoptotic cell appears as a round or oval mass with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments.

Electron microscopy is the best way to characterize the subcellular changes. Early during the chromatin condensation phase, the electron-dense nuclear material aggregates peripherally under the nuclear membrane although there can also be uniformly dense nuclei. Extensive plasma membrane blebbing occurs followed by karyorrhexis and separation of cell

fragments into apoptotic bodies during a process called budding. Apoptotic bodies consist of cytoplasm with tightly packed organelles with or without a nuclear fragment. The organelle integrity is still maintained and all of this is enclosed within an intact plasma membrane. These bodies are subsequently phagocytosed by macrophages, parenchymal cells, or neoplastic cells and degraded within phagolysosomes. There is essentially no inflammatory reaction associated with the process of apoptosis nor with the removal of apoptotic cells because apoptotic cells do not release their cellular constituents into the surrounding interstitial tissue. Instead, they are quickly phagocytosed by surrounding cells thus likely preventing secondary necrosis; and the engulfing cells do not produce anti-inflammatory cytokines (Savill and Fadok, 2000; Kurosaka et al., 2003).

**Mechanism**

The mechanisms of apoptosis are highly complex and involve an energy-dependent cascade of molecular events. There are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. However, these pathways can be linked and each can influence the other (Igney and Krammer, 2002; Elmore, 2007).

***Extrinsic Pathway***

The extrinsic signaling pathway that initiates apoptosis involves transmembrane receptor-mediated interactions. These involve death receptors that are members of the TNF receptor gene superfamily (Locksley et al., 2001). The most well-known ligands and corresponding death receptors include FasL/FasR, TNFα/TNFR1, Apo3L/DR3, Apo2L/DR4 and Apo2L/DR5 (Chicheportiche et al., 1997; Ashkenazi and Dixit, 1998; Peter and Krammer, 1998; Suliman et al., 2001; Rubio-Moscardo et al., 2005). The binding of Fas ligand to Fas receptor results in the binding of the adapter protein Fas-Associated Protein

with Death Domain (FADD) and the binding of TNF ligand to TNF receptor results in the binding of the adapter protein Tumor necrosis factor receptor type 1-associated DEATH domain protein (TRADD) with recruitment of FADD and RIP (Hsu et al., 1995; Grimm et al., 1996; Wajant et al., 2002). FADD then associates with procaspase-8 via dimerization of the death effector domain. At this point, DISC is formed, resulting in the autocatalytic activation of procaspase-8 (Kischkel et al., 1995). Once caspase-8 is activated, the execution phase of apoptosis is triggered. Death receptor-mediated apoptosis can be inhibited by a protein called c-FLIP which will bind to FADD and caspase-8, rendering them ineffective (Kataoka et al., 1998; Scaffidi et al., 1999).

***Intrinsic Pathway***

The intrinsic signaling pathway involves non-receptor mediated stimuli that produce intracellular signals that act directly on targets within the cell and are mitochondrial initiated events. Examples include radiation, toxins, hypoxia, hyperthermia, viral infections and free radicals.

All of these stimuli cause changes in the inner mitochondrial membrane that result in loss of the mitochondrial transmembrane potential and release of normally sequestered pro-apoptotic proteins from the intermembrane space into the cytosol (Saelens et al., 2004). Cytochrome *c*, Smac/DIABLO, and the serine protease HtrA2/Omi (Cai et al., 1998, Du et al., 2000, van Loo et al., 2002) activate the caspase-dependent mitochondrial pathway. Cytochrome *c* binds and activates Apaf-1 as well as procaspase-9, forming an apoptosome (Hill et al., 2004; Chinnaiyan, 1999). The clustering of procaspase-9 in this manner leads to caspase-9 activation. Smac/DIABLO and HtrA2/Omi are reported to promote apoptosis by

inhibiting inhibitors of apoptosis (IAP) protein activity (van Loo et al., 2002; Schimmer, 2004).

The control of these apoptotic mitochondrial events occurs through members of the Bcl-2 family of proteins (Cory and Adams, 2002). They control mitochondrial membrane permeability and can be either pro-apoptotic or anti-apoptotic. Twenty-five genes have been identified in the Bcl-2 family. Some of the anti-apoptotic proteins include Bcl-2, Bcl-x, Bcl-XL, Bcl-XS, Bcl-w, BAG, and some of the pro-apoptotic proteins include Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, and Blk. It is thought that the main mechanism of action of the Bcl-2 family of proteins is the regulation of cytochrome *c* release from the mitochondria via alteration of mitochondrial membrane permeability.

***The Executioner Pathway***

The extrinsic and intrinsic pathways both converge at the point of the execution phase, considered the final pathway of apoptosis. Execution caspases activate cytoplasmic endonuclease, which degrades nuclear material, and proteases that degrade the nuclear and cytoskeletal proteins. Caspase-3, caspase-6, and caspase-7 function as effector or executioner caspases, cleaving various substrates that ultimately cause the morphological and biochemical changes seen in apoptotic cells (Slee et al., 2001).

Caspase-3 is considered to be the most important of the executioner caspases and is activated by any of the initiator caspases (caspase-8, caspase-9, or caspase-10). Caspase-3 specifically activates the endonuclease Caspase-activated DNase (CAD). In proliferating cells CAD is complexed with its inhibitor, ICAD. In apoptotic cells, activated caspase-3 cleaves ICAD to release CAD (Sakahira et al., 1998). CAD then degrades chromosomal DNA within the nuclei and causes chromatin condensation. Caspase-3 also induces cytoskeletal reorganization and disintegration of the cell into apoptotic bodies.

Phagocytic uptake of apoptotic cells is the last component of apoptosis. Phospholipid asymmetry and externalization of phosphatidylserine on the surface of apoptotic cells and their fragments is the hallmark of this phase. Although the mechanism of phosphatidylserine translocation to the outer leaflet of the cell during apoptosis is not well understood, it has been associated with loss of aminophospholipid translocase activity and nonspecific flip-flop of phospholipids of various classes (Bratton et al., 1997).

### Rationale

The survival of differentiation-competent adipose progenitor cells may influence the mechanism by which AT expands, in a depot-specific manner. The implication of macrophage-secreted factors in the regulation of adipose progenitor cell differentiation and proliferation suggests that adipose progenitor cell survival may also be affected. Adipose progenitor cell survival through the inhibition of apoptosis would maintain a population of progenitors capable of expanding the adipocyte pool through adipogenesis. In contrast, loss of adipose progenitors through apoptosis would contribute to an adipogenic deficit, predisposing to excess adipocyte hypertrophy and dysfunctional AT.

## Hypothesis

Depot origin influences susceptibility of adipose progenitor cells to apoptosis.

### Objectives

***Objective 1:*** *To compare cell death between SC and OM progenitor cells in response to various apoptotic stimuli.*

***Objective 2:*** *To evaluate the effect of macrophage-secreted factors on the cell death of SC versus OM progenitor cells.*

## 2.0 Materials and Methods

### Cell Model used for Objectives

#### *Human Primary Adipose Progenitor Cells*

Human primary adipose progenitor cells were isolated by size fractionation and centrifugation from the SVF of digested SC and OM AT matched from the same individual (Skurt and Hauner, 2012). They possess a fibroblast-like morphology and are not completely defined with respect to cell surface protein expression (Decaunes et al., 2011; Zimmerlin et al., 2010). The human adipose progenitor cell is a relevant cell model; however variability exists between cell responses due to donor heterogeneity. Human primary adipose progenitor cells that undergo excessive proliferation through excessive passaging eventually lose the ability to differentiate.

#### *Monocyte-Derived Macrophages*

MD-macrophages are isolated from the peripheral blood mononuclear cell (PBMC) layer after Ficoll-gradient centrifugation of whole blood (Johnson et al., 1977). Following seeding into culture dishes, adherent monocytes differentiate into macrophage phenotype. MD-macrophages can be activated towards pro- or anti-inflammatory phenotypes with different cytokine treatments; basally, MD-macrophages are mildly skewed towards an anti-inflammatory activation state (Daigneault et al., 2010; Martinez et al., 2006). MD-macrophages are a useful primary human macrophage cell model; however donor-related experimental variability may be a factor.

### *Inducers of Apoptosis*

We stimulated apoptosis in our human adipose progenitor cells by serum deprivation, serum deprivation with 438 ng/mL recombinant human TNFα (Niesler et al., 1998), or with a combination of 10 ng/mL TNFα and 10 mg/mL CHX (Molgat et al., 2012).

## Isolation and Culture of Human Adipose Progenitor Cells

SC and OM AT samples were obtained or matched from the same patients undergoing elective abdominal surgery (approved by the Ottawa Health Science Network Research Ethics Board, OHSN-REB). Subsets of patients (3-5), from the pool of twenty (12 female and 8 male), were used for each individual study. Mean age (±SD) was 49.7 ± 2.2 years, and mean BMI was 28.6 ± 0.7 kg/m$^2$. The inclusion criteria were as follows: all patients were between 18 and 70 years of age, no patients had diabetes, no patients were on glucocorticoids and none underwent dramatic changes in weight within six months prior to surgery. Adipose progenitor cells were isolated as described (Artemenko et al., 2005). AT samples (2-15 g) were received in a disposable sterile plastic specimen bottle with cold DMEM/F12 media with 2X antibiotics. Samples were rinsed with PBS to remove blood. Fibrous material, such as connective tissue and capillaries, were removed from AT by dissection and discarded. The remaining tissue was cut into small pieces, weighed and digested with collagenase CLS type I to isolate individual cells from matrix proteins without damaging the cells and influencing their growth potential. The first digestion was carried out for 30-45 minutes at 37$^o$C in a shaking incubator. The digested tissue was subjected to progressive size filtration through a nylon mesh (250 μM). The remaining tissue was digested (30 minutes) and filtered once again. The cell suspension was centrifuged for 10 minutes at 200 g, and then incubated in an erythrocyte lysis buffer for 10 minutes at room

temperature. After several washing and centrifugation steps, the floating mature adipocytes were aspirated. The SVF was re-suspended in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and the cells were counted using the Neubauer hemocytometer. Adipose progenitor cells were seeded at 2.5 x $10^5$-5.0 x $10^5$ in 100 mm tissue culture dishes. Adipose progenitor cells were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 50 U/ml nystatin (Calbiochem, Merck KGaA; Darmstadt, Germany), and grown until confluent.

**Preparation of Macrophage Conditioned Medium**

To prepare MacCM from human MD-macrophages, PBMCs were isolated from blood donated by healthy volunteers (two female and two male; mean age (±SD) was 23 ± 0.4 years; approved by the OHSN-REB). Collected blood (120 mL) was pooled into a 500 ml bottle and diluted 2:1 with PBS + 2 mM EDTA (two part 1x PBS, one part whole blood). Centrifuge tubes were filled with 15 ml Ficoll-Hypaque (GE Healthcare; Uppsala, Sweden), and layered with 30 ml diluted blood. Blood was centrifuged (520 x g) for 30 minutes. The top serum (yellow) layer was discarded and the interphase containing the PBMCs (white layer) was collected and placed in a new centrifuge tube. The tube was filled with PBS + 2 mM EDTA and centrifuged at 520 x g for five minutes. The PBS was discarded and the wash and centrifugation step was repeated. The PBS was discarded and the pellet was resuspended in 3 mL of RPMI supplemented with 100 U/ml penicillin and 0.1 mg/ml streptomycin. Cells were counted using the Neubauer hemocytometer. PBMCs were seeded at a density of 1 x $10^6$ cells/well in 12 well culture dishes. Monocytes were allowed to adhere for 1 h in serum-free RPMI, and they were subsequently differentiated into macrophages over 7 days in RPMI supplemented with 10% FBS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin {{316

Daigneault,M. 2010; 320 Mayi,T.H. 2010}}. Differentiated human macrophages were exposed to RPMI supplemented with 100 U/ml penicillin, and 0.1 mg/ml streptomycin (1 mL/well) and collected 24 h later (Franco et al., 2006). MacCM and control media (RPMI with 100 U/ml penicillin, and 0.1 mg/ml streptomycin) were subjected to the same experimental conditions.

## Cell Death Analysis

### *Cell Enumeration*

Human adipose progenitor cells were grown on 35 mm dishes until confluent. The confluent human adipose progenitor cells (2.5 x $10^5$/35 mm dish) were placed in medium supplemented with 10% FBS, serum-free, serum-free with TNFα (438 ng/mL) alone, or with a combination of TNFα (10 ng/mL) and CHX (10 µg/mL), for 24 h. After 24 h, floating cells were removed by aspiration, and adherent cells were trypsinized using 0.5 ml trypsin. Each dish was incubated at 37$^o$C for 5 minutes. Once all the cells lifted, 0.5 ml of DMEM supplemented with 20% calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin were added to each dish and the cells were transferred to a microfuge tube. Once in the microfuge tube, the cells were suspended by gently pipetting up and down three times, and a 10 µl aliquot was placed on either side of the Neubauer hemocytometer. Viable cells were counted in duplicate using a using a light microscope. Each treatment was performed in duplicate.

### *Hoechst Staining*

Primary human adipose progenitor cells were seeded at 2.5 x $10^5$/35 mm dish and grown on coverslips. Cells were placed in control medium (supplemented with 10% FBS), serum-free, serum-free with TNFα (438 ng/mL), or a combination of TNFα (10 ng/mL) with

CHX (10 µg/mL), for 6 h to induce apoptosis. In the macrophage studies, the apoptotic conditions for the adipose progenitor cells remained the same except cells were treated with MacCM or control media. The cells were washed three times with 1 ml PBS. Cells were fixed in 1 ml of 10% formalin (formaldehyde) for 1 h, and then washed twice with 1 ml PBS. The coverslips were removed and placed face up in the humid chamber. The cells were stained for 10 min with 50 µl of Hoechst staining solution (1 µg/ml). Individual coverslips were washed in three consecutive beakers containing PBS and one containing water. The coverslips were mounted face down onto glass slides using 15 µl of Moviol, and set to dry overnight in the dark. Cells were visualized and photographed (400 x mag) with a Zeiss Axio Imager.M1 microscope equipped with an Axiocam HRm digital camera (Carl Zeiss, Toronto, Canada). The number of cells counted was >400 cells per treatment. Percent apoptosis was calculated by dividing the total number of apoptotic nuclei by the total number of nuclei (counted by two independent observers, blinded to the conditions), multiplied by 100.

**Statistical Analysis**

Experiments were performed on cells isolated from at least 3 subjects. Comparison of means was performed by two-way ANOVA (with replication) and $P<0.05$ was taken as significant. The Pearson Correlation was the statistical method used for all correlation analyses.

## 3.0 Results

### Depot Differences in Adipose Progenitor Cell Death

Cell death was measured using Hoechst staining of condensed nuclei and cell enumeration. Adipose progenitor cells were treated for 6 and 24 h when assessed using Hoechst staining or cell counting, respectively.

### Results for Aim 1

As expected (Fig. 1-14), no matter which of the three apoptotic conditions that were examined, apoptosis of adipose progenitor cells cultured in serum-containing medium was very low (<1%). No difference was found in this basal apoptotic index between SC and OM adipose progenitor cells.

#### *Effect of Serum Deprivation on the Cell Death of Adipose Progenitor Cells*

The effect of serum deprivation on the apoptosis of abdominal SC and OM adipose progenitor cells was assessed by Hoechst staining and expressed as the mean apoptotic index (% ± SE). The SC depot showed minor apoptosis that was not significant (Fig. 1). The OM depot displayed significant apoptosis compared to the control (3.4 ± 0.7 vs 0.65 ± 0.17) ($P$<0.01). Apoptosis was significantly greater in the OM depot than in the SC depot. OM adipose progenitor cells displayed a mean apoptotic index of 3.4 ± 0.7, whereas SC adipose progenitor cells demonstrated a mean index of 1.2 ± 0.1 ($P$ < 0.05).

There was a significant effect of donor BMI on the relative apoptotic susceptibility between the two types of adipose progenitors (Fig. 2). The same patient samples were used as in Fig. 1. The patient samples with the highest ratios of OM/SC % apoptosis also had the

highest BMIs (R=0.9435; *P*=0.05), with the apoptosis response of the OM versus SC adipose progenitor cells being BMI dependent.

As assessed by cell enumeration, each depot had minor percent cell death that was not significant, and there was no depot difference (Fig. 3). Serum withdrawal resulted in a trend of greater percent cell death in the OM depot (30.0 ± 4.0) compared to the SC depot (19.0 ± 4.0), but did not reach significance (*P*=0.12).

We did not observe a significant depot difference; however there was a significant effect of BMI of the donors on the relative death susceptibility between the two types of adipose progenitors (Fig. 4). As previously observed with Hoechst staining, OM/SC % cell death positively correlated to the patient BMIs (R=0.9590; *P*=0.0099). The same patient samples were used as in Fig. 3.

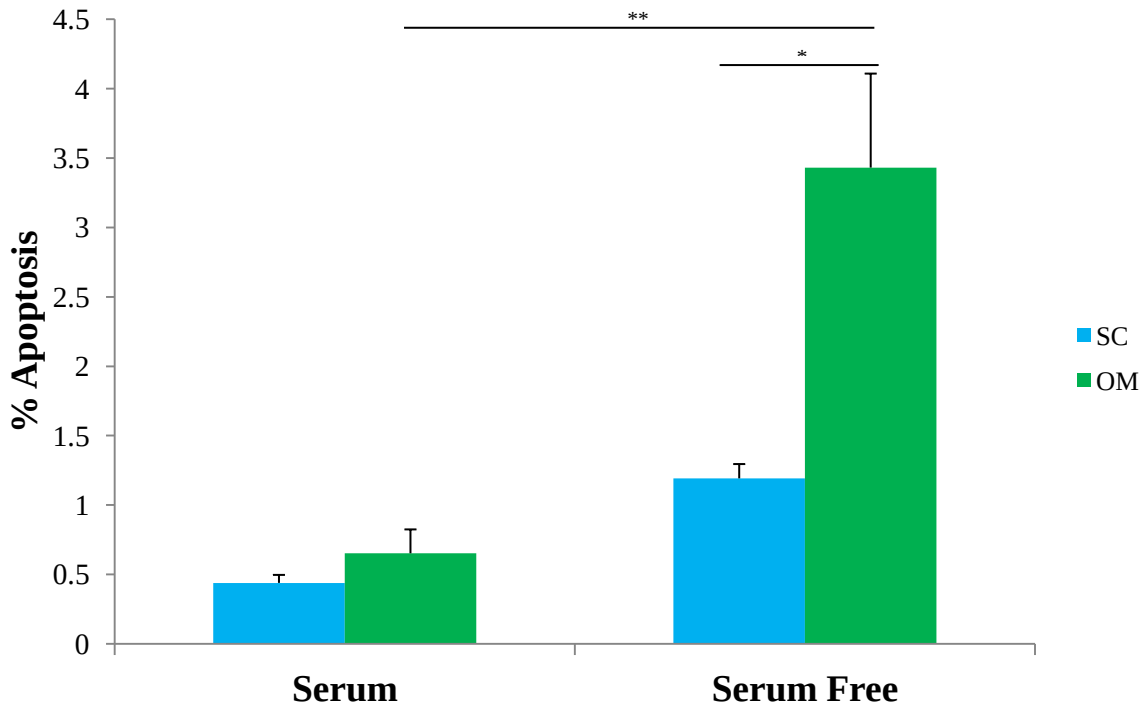


**Fig. 1 Primary human adipose progenitor cell apoptosis induced by serum deprivation.** Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free media for 6 h. Cells were fixed then stained with Hoechst dye. Staining was visualized by fluorescence. Hoechst staining was performed on duplicate dishes. Results are the mean ± SE of 4 separate matched patient samples. *P<0.05, **P<0.01.

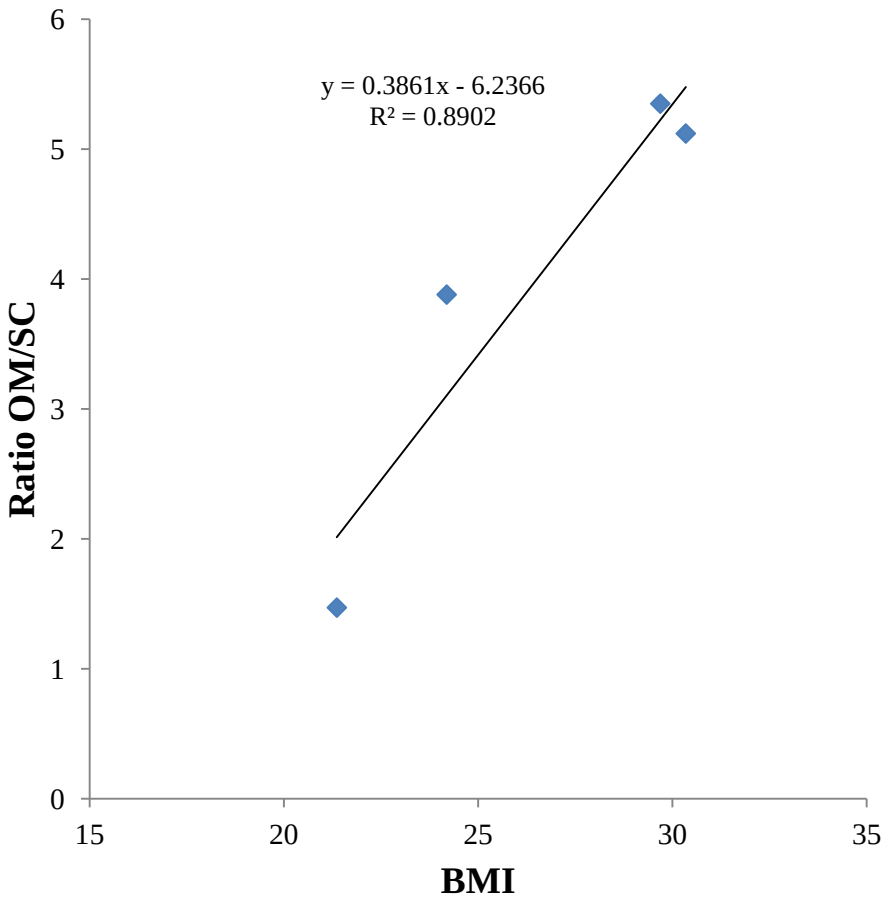


**Fig. 2 Apoptosis induced by serum deprivation correlates with BMI.** Scatter plot shows the BMI correlation of the 4 donors on the relative apoptotic susceptibility between the two types of adipose progenitors. P = 0.0546. Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium for 6 h. Cells were fixed then stained with Hoechst dye. Staining was visualized by fluorescence. Hoechst staining was performed on duplicate dishes. Results are the mean+/-SE of 4 separate matched patient samples.

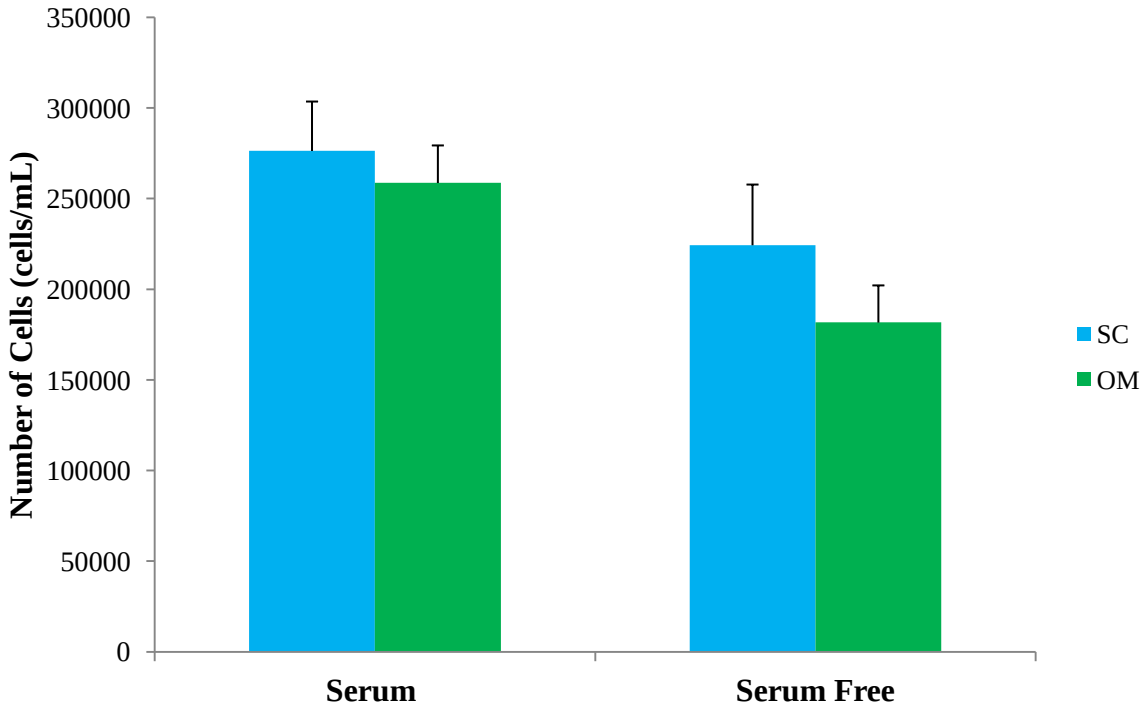


**Fig. 3 Primary human adipose progenitor cell death induced by serum deprivation.** Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium for 24 h. Cell enumeration was performed on duplicate or triplicate dishes. Results are the mean+/-SE of 5 separate matched patient samples and are not significant.

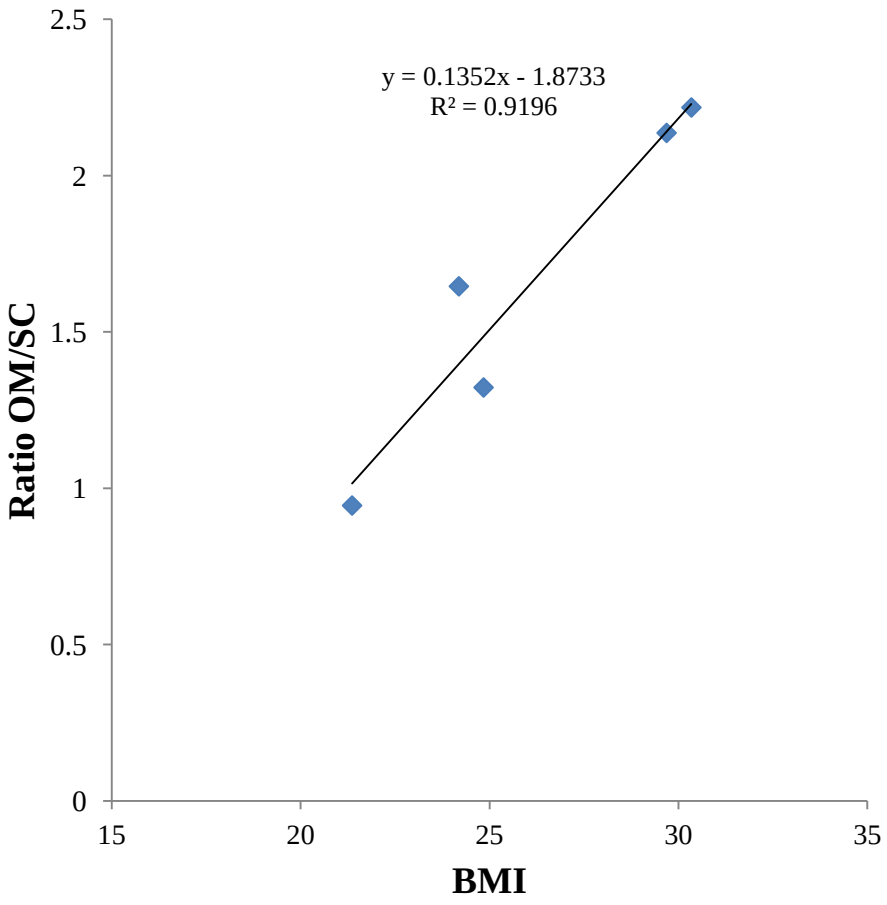


**Fig. 4 Cell death induced by serum deprivation correlates with BMI.** Scatter plot shows the BMI correlation of the 5 donors on the relative death susceptibility between the two types of adipose progenitors. P = 0.0099. Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium for 24 h. Cell enumeration was performed on duplicate or triplicate dishes. Results are the mean+/-SE of 5 separate matched patient samples.

### *Effect Serum Deprivation and TNFα on the Cell Death of Adipose Progenitor Cells*

The effect of serum deprivation and TNFα on the apoptosis of abdominal SC and OM adipose progenitor cells was assessed by Hoechst staining and represented as the mean apoptotic index (% ± SE). The SC depot showed significant apoptosis compared to the control (3.1 ± 0.5 vs 0.5 ± 0.1) ($P$<0.01) (Fig. 5). The OM depot also displayed significant apoptosis compared to the control (0.6 ± 0.1 vs 5.1 ± 0.2) (P<0.01). Apoptosis was significantly greater in the OM depot than in the SC depot. OM adipose progenitor cells displayed a mean apoptotic index of 5.1 ± 0.2, whereas SC adipose progenitor cells demonstrated a mean index of 3.1 ± 0.5 ($P$ < 0.01). TNFα increased the level of apoptosis beyond that observed in serum-free medium alone.

In contrast to serum deprivation alone, there was a negative effect of BMI of the donors on the relative apoptotic susceptibility between the two types of adipose progenitors (Fig. 6). The same patient samples were used as in Fig. 5. The patient sample ratios of OM/SC % apoptosis negatively correlated with their BMIs (R=0.9997; $P$=0.0099).

Cell enumeration revealed that each depot had significant percent cell death compared to its respective control ($P$<0.01) (Fig. 7). OM adipose progenitor cells underwent significantly more percent cell death (40.0 ± 2.0) versus cells from the SC depot (28.0 ± 0.2) (P<0.01). Cell death induced by serum deprivation with TNFα did not correlate with BMI (Fig. 8). The same patient samples were used as in Fig. 7.

### *Effect of Serum Deprivation and TNFα/CHX on the Cell Death of Adipose Progenitor Cells*

When TNFα in combination with CHX was added to the serum-free medium, Hoechst staining revealed that both SC and OM depots underwent significant apoptosis (14.8

± 4.3 versus 11.9 ± 2.8, P<0.01) (Fig. 9). However, no depot difference was observed under these conditions. Apoptosis induced by serum deprivation with TNFα and CHX did not correlate with BMI (Fig. 10). The same patient samples were used as in Fig. 9.

Cell enumeration also showed that each depot had significant percent cell death compared to its respective control (*P*<0.01) (Fig. 11). However, there was no depot difference in percent cell death (44.0 ± 4.0 versus 43.0 ± 3.0) (P=0.878). Similarly, cell death induced by serum deprivation with TNFα and CHX did not correlate with BMI (Fig. 12). The same patient samples were used as in Fig. 11.

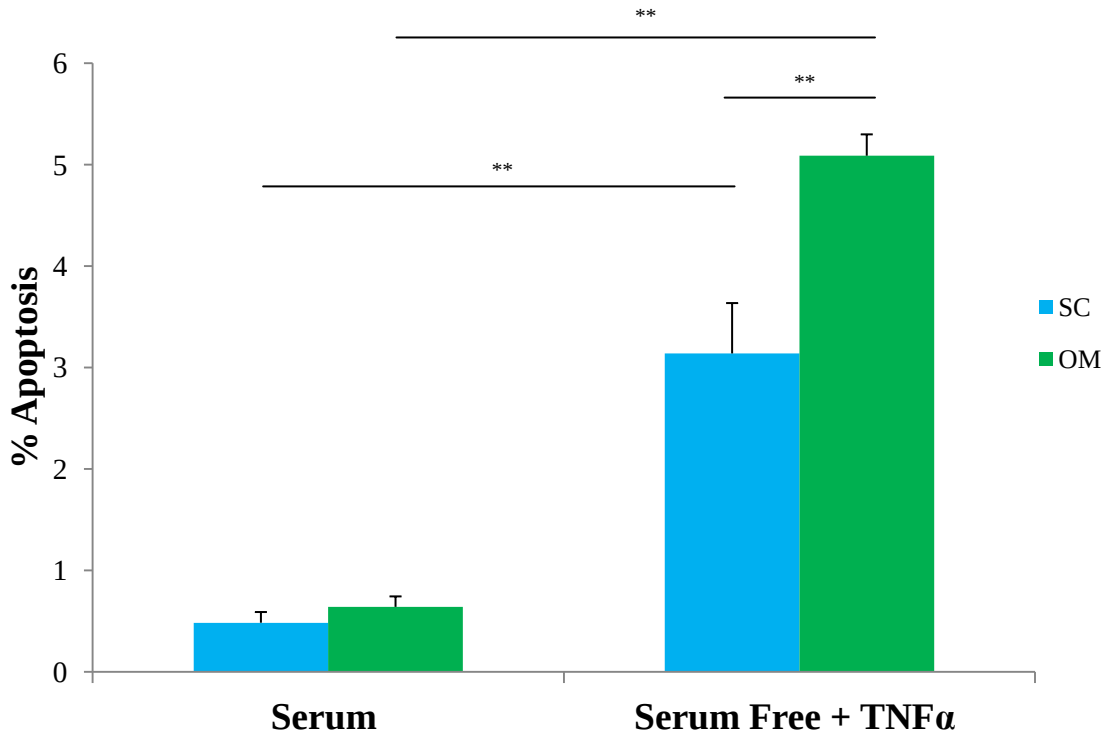


**Fig. 5 Primary human adipose progenitor cell apoptosis induced by serum deprivation with TNFα.** Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 438 ng/mL of TNFα for 6 h. Cells were fixed then stained with Hoechst dye. Staining was visualized by fluorescence. Hoechst staining was performed on duplicate dishes. Results are the mean ± SE of 3 separate matched patient samples. **P<0.01.

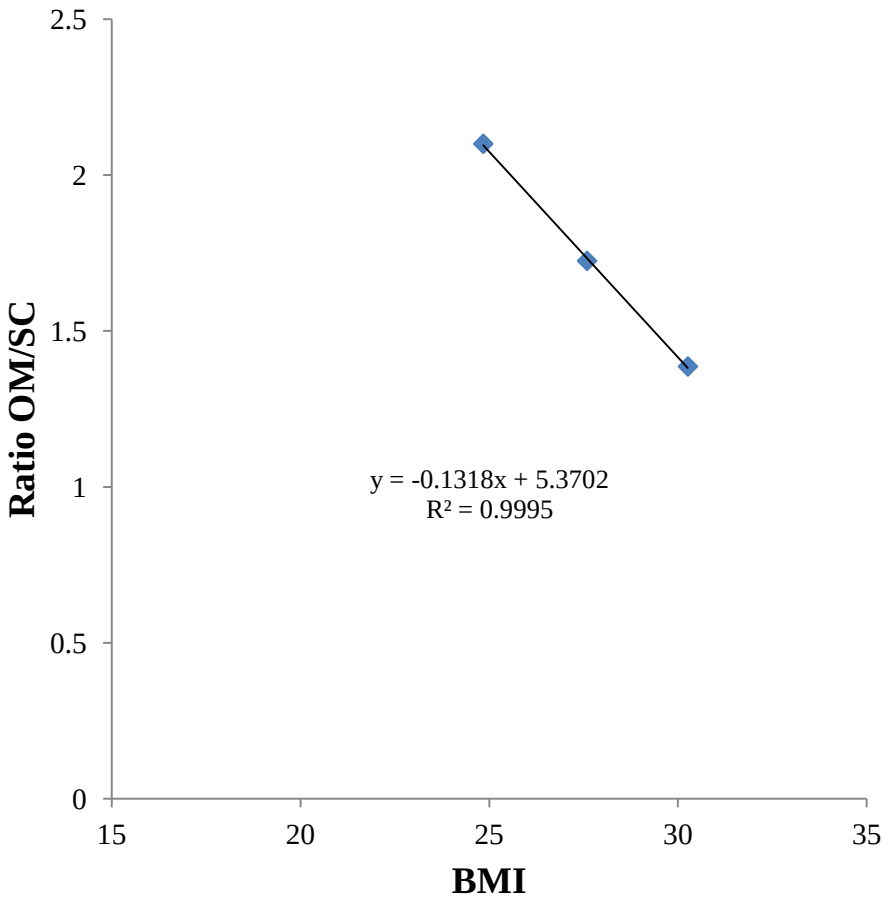


**Fig. 6 Apoptosis induced by serum deprivation with TNFα correlates negatively with BMI.** Scatter plot shows the correlation BMI of the 3 donors on the relative apoptotic susceptibility between the two types of adipose progenitors. P = 0.0099 Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 438 ng/mL of TNFα for 6 h. Cells were fixed then stained with Hoechst dye. Staining was visualized by fluorescence. Hoechst staining was performed on duplicate dishes. Results are the mean+/-SE of 3 separate matched patient samples.

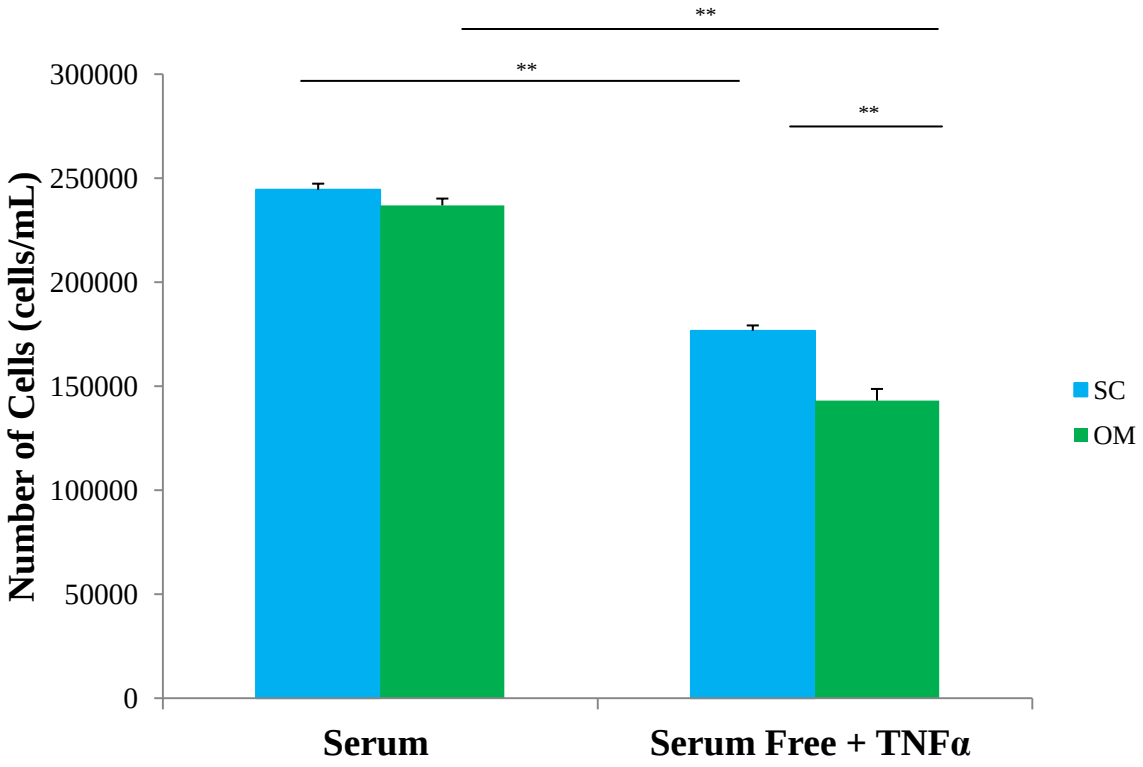


**Fig. 7 Primary human adipose progenitor cell death induced by serum deprivation with TNFα.** Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 438 ng/mL of TNFα for 24 h. Cell enumeration was performed on duplicate or triplicate dishes. Results are the mean+/-SE of 3 separate matched patient samples. **P<0.01.

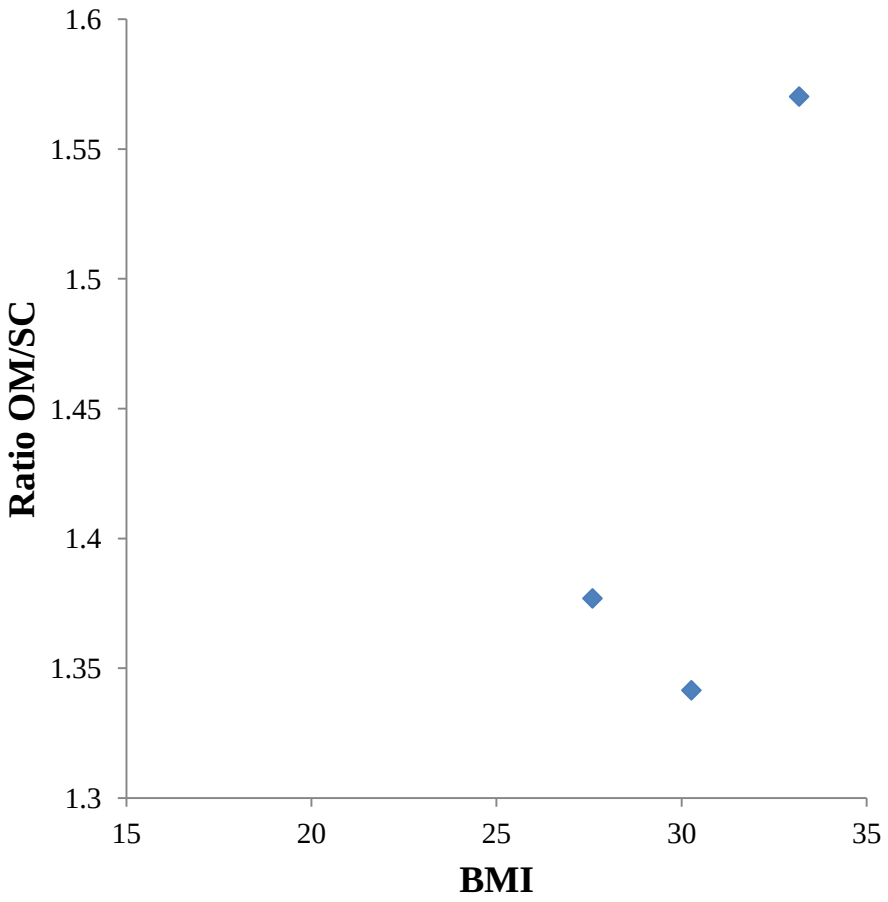


**Fig. 8 Cell death induced by serum deprivation with TNFα does not correlate with BMI**. Scatter plot shows the BMI correlation of the 3 donors on the relative death susceptibility between the two types of adipose progenitors. The data are not significant. Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 438 ng/mL of TNFα for 24 h. Cell enumeration was performed on duplicate or triplicate dishes. Results are the mean+/-SE of 3 separate matched patient samples.

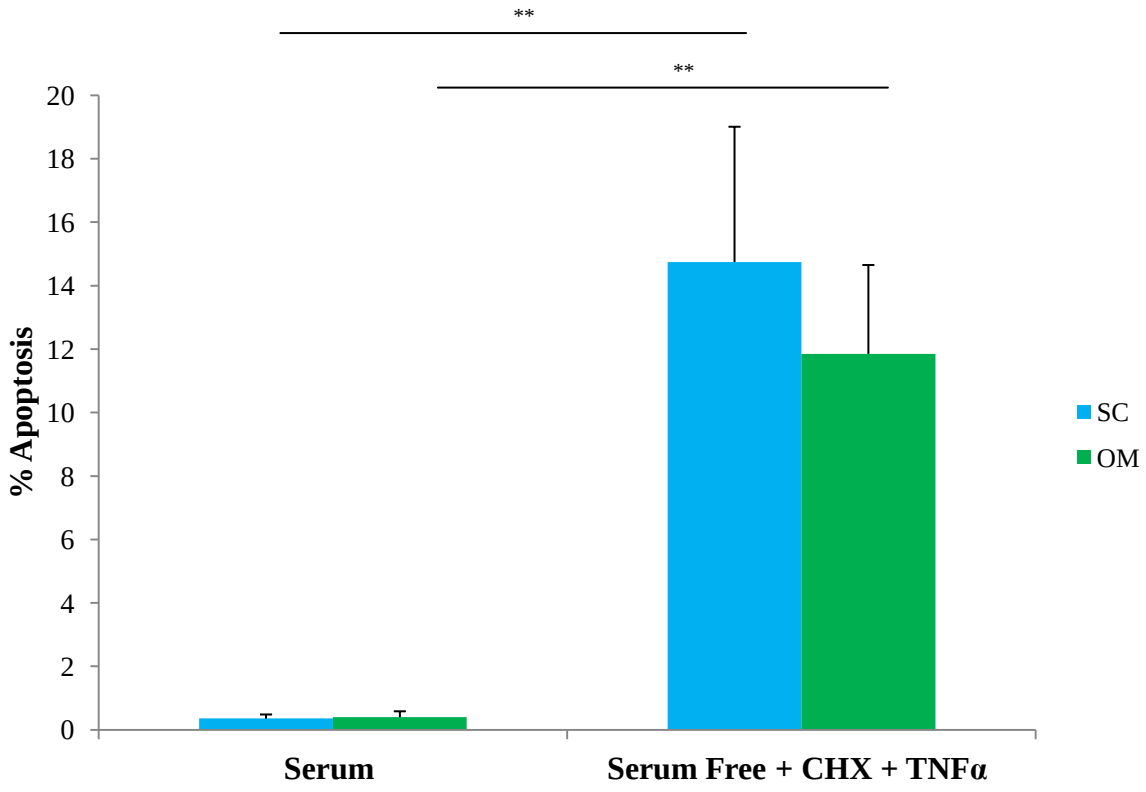


**Fig. 9 Primary human adipose progenitor cell apoptosis induced by serum deprivation with TNFα and CHX.** Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 10 ng/mL of TNFα and 10 μg/mL CHX for 6 h. Cells were fixed then stained with Hoechst dye. Staining was visualized by fluorescence. Hoechst staining was performed on duplicate dishes. Results are the mean+/-SE of 3 separate matched patient samples. **P<0.01.

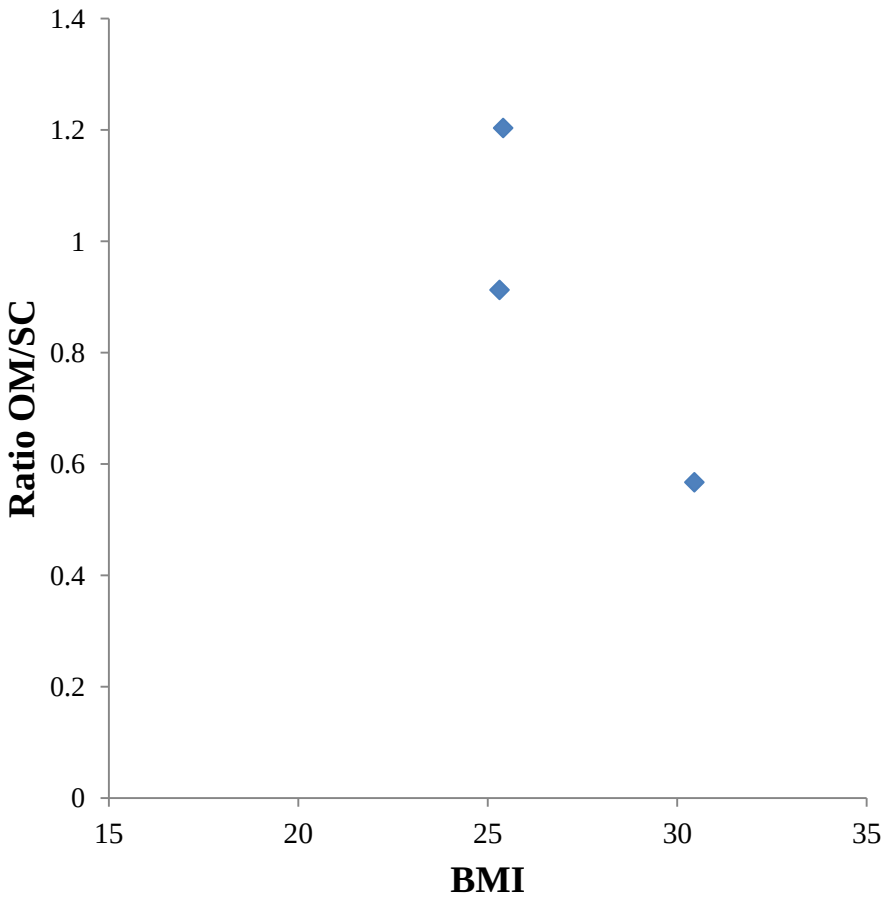


**Fig. 10 Apoptosis induced by serum deprivation with TNFα and CHX does not correlate with BMI.** Scatter plot shows the BMI correlation of the 3 donors on the relative apoptotic susceptibility between the two types of adipose progenitors. The data are not significant. Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 10 ng/mL of TNFα and 10 μg/mL CHX for 6 h. Cells were fixed then stained with Hoechst dye. Staining was visualized by fluorescence. Hoechst staining was performed on duplicate dishes. Results are the mean+/-SE of 3 separate matched patient samples.

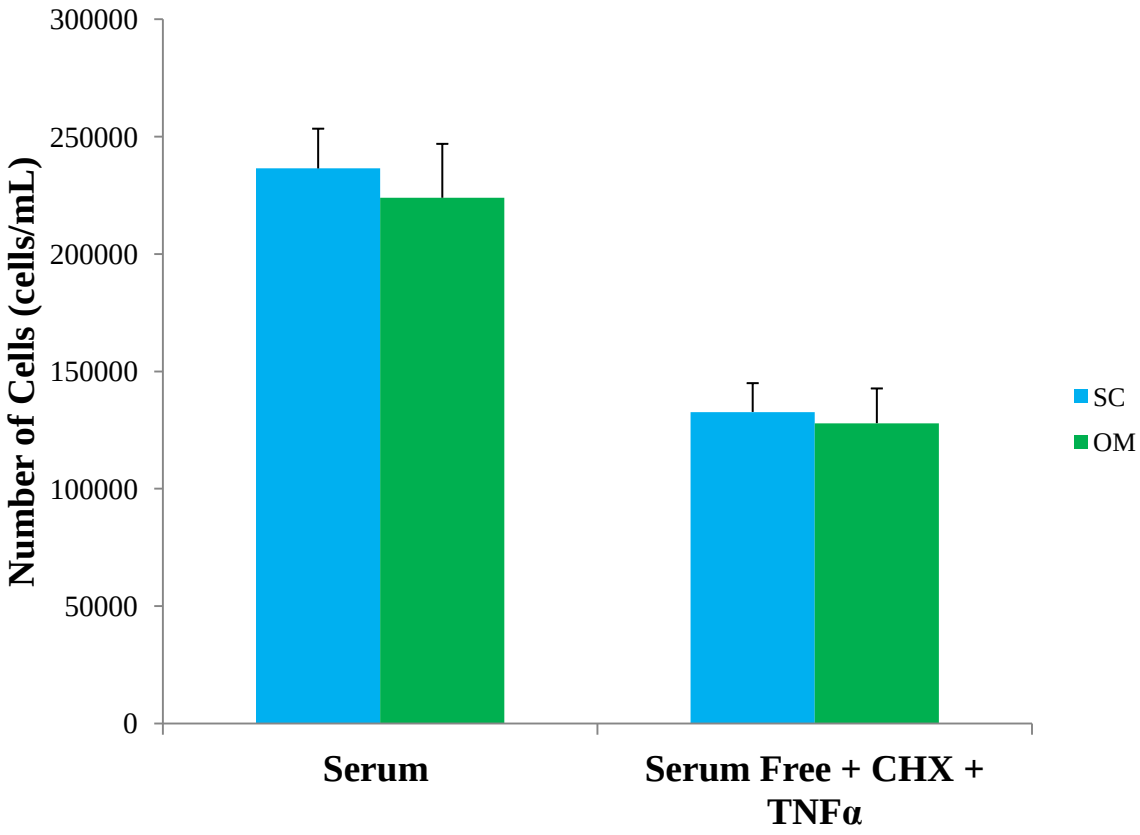


**Fig. 11 Primary human adipose progenitor cell death induced by serum deprivation with TNFα and CHX.** Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 10 ng/mL TNFα and 10 μg/mL CHX for 24 h. Cell enumeration was performed on duplicate or triplicate dishes. Results are the mean+/-SE of 4 separate matched patient samples. **P<0.01.

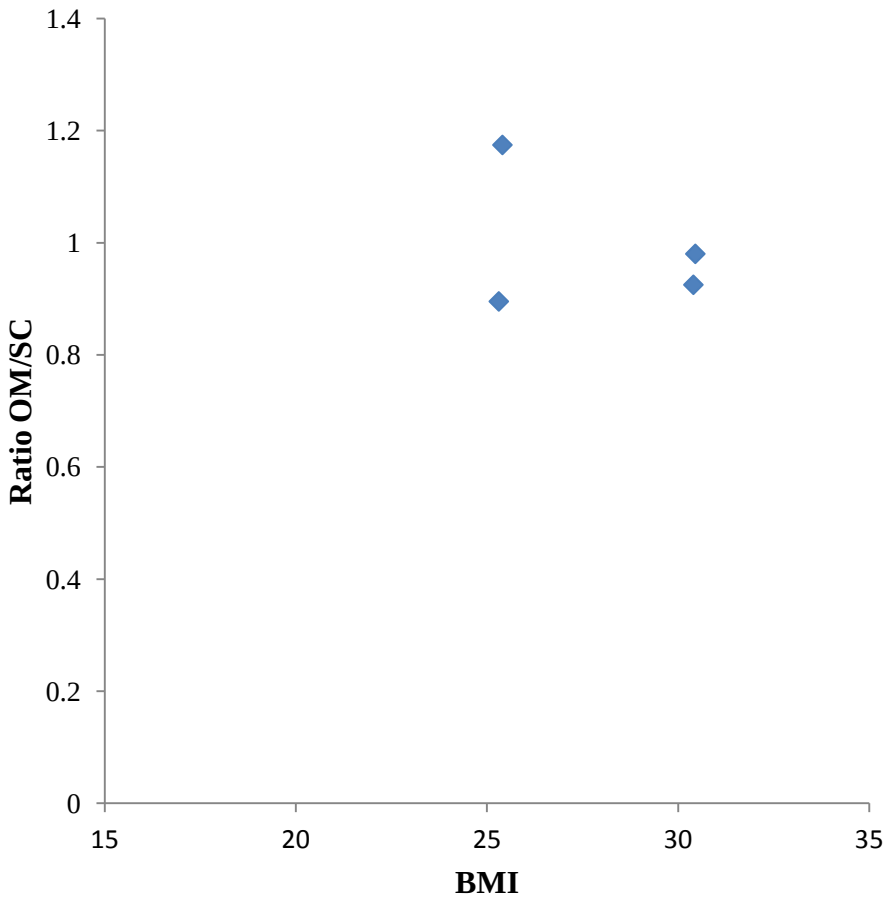


**Fig. 12 Cell Death induced by serum deprivation with TNFα and CHX does not correlate with BMI.** Scatter plot shows the BMI correlation of the 4 donors on the relative cell death susceptibility between the two types of adipose progenitors. The data are not significant. Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 10 ng/mL TNFα and 10 μg/mL CHX for 24 h. Cell enumeration was performed on duplicate or triplicate dishes. Results are the mean+/-SE of 4 separate matched patient samples.

### *Optimal Detection of Cell Death Occurs at 6 and 24 h as Assessed by Hoechst Staining and Cell Enumeration under Conditions of Serum Deprivation and TNFα*

Time course analyses were performed to assess whether 6 and 24 h treatment periods were optimal to detect cell death. Hoechst staining and cell enumeration were used to assess cell death under conditions of serum deprivation with TNFα. Additionally, I wanted to see if my current observations occurred at other time points.

Hoechst staining was conducted on cells treated with TNFα for 1 h, 4 h, 18 h, 24 h and 48 h (Fig. 13). A significant difference was found in the apoptotic index between treated SC (2.5 ± 0.6) and OM (8.9 ± 0.4) adipose progenitors ($P<0.01$) at 4 h. The OM depot showed a trend of greater apoptosis compared to the SC depot at 18 h (SC, 1.7 ± 0.4; OM, 7.9 ± 1.5) and 24h (SC, 2.9 ± 1.0; OM, 6.9 ± 1.8), however this was not significant. After the 48 h treatment time, apoptosis appeared to decrease in the OM depot, eliminating any depot difference. I concluded that a treatment period of 4 to 6 h was optimal to assess apoptosis using the Hoechst staining.

Cell enumeration was used to assess cell death after serum deprivation and TNFα treatment for 24 and 48 h. Although both showed increased percent death rates, treatment for 24 h resulted in a trend of greater cell death in the OM depot (43.4 ± 3) compared to the SC depot (36.2 ± 3), but did not reach significance ($P=0.12$) (Fig. 14). However, a significant depot difference was observed at 48 h (SC, 39.6 ± 2%; OM, 55.9 ± 4 ($P<0.05$). I concluded that a treatment period of 24 to 48 h was optimal to cause cell death as assessed by cell enumeration.

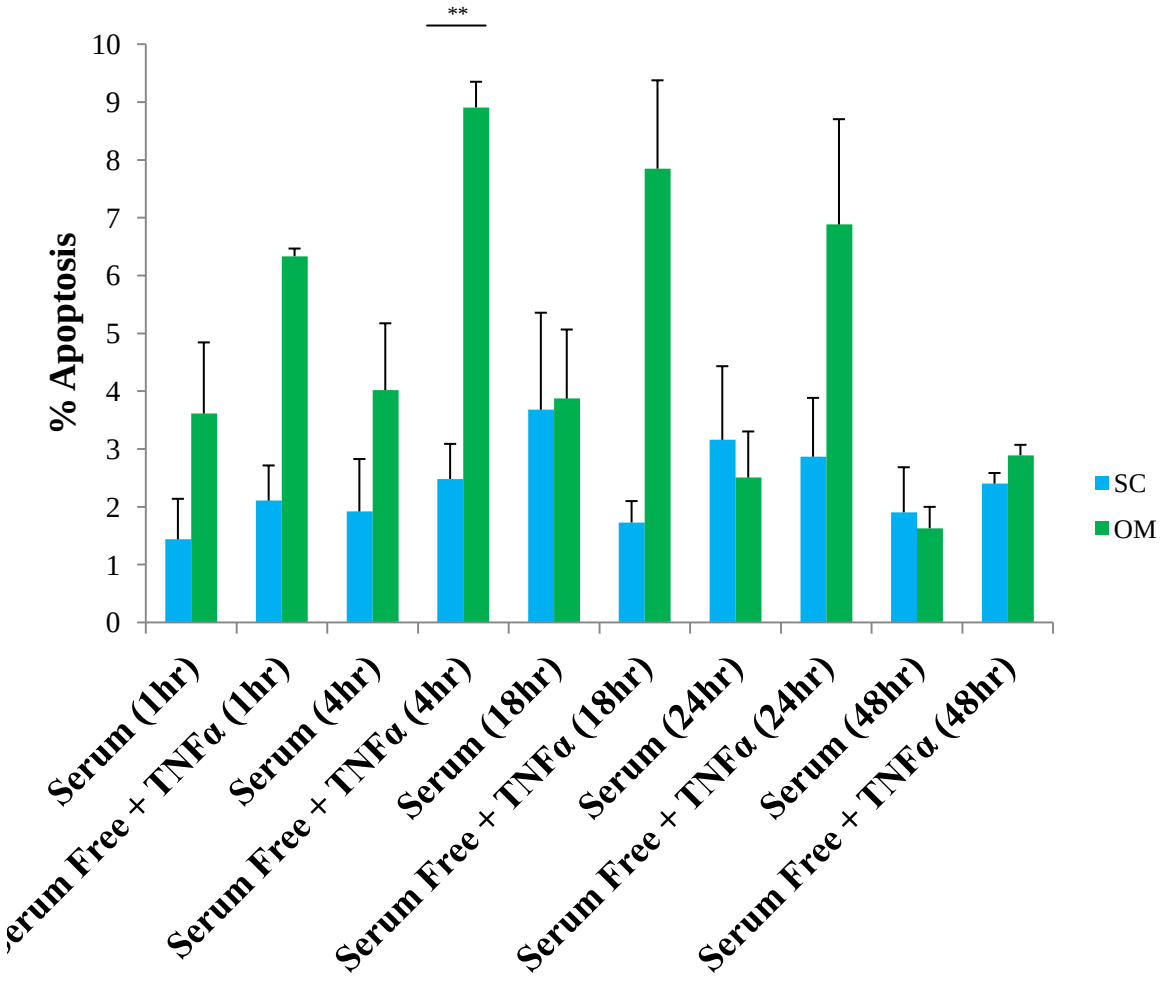


**Fig. 13 Serum deprivation with TNFα for 6 h is sufficient to induce primary human adipose progenitor cell apoptosis.** Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 438 ng/mL of TNFα for 1 h, 4 h, 18 h, 24 h and 48 h. Cells were fixed then stained with Hoechst dye. Staining was visualized by fluorescence. Hoechst staining was performed on duplicate dishes. Results are the mean+/-SE of 3 separate matched patient samples. **P<0.01.

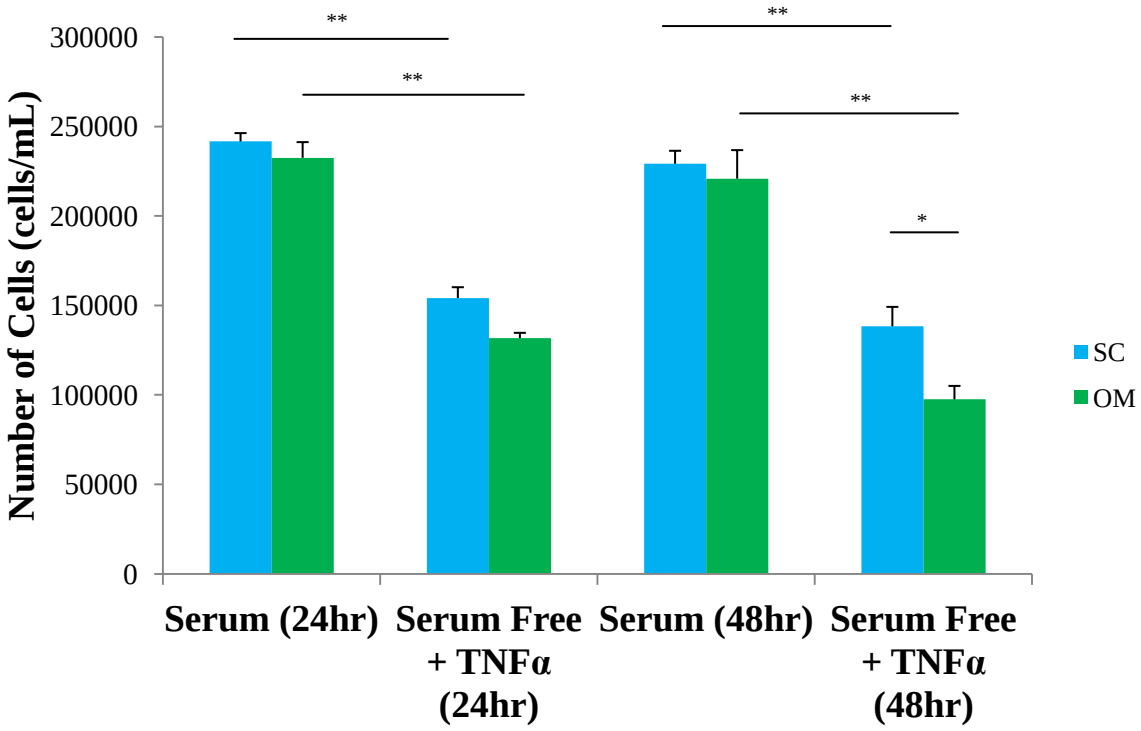


**Fig. 14 Serum deprivation with TNFα for 24 h is sufficient to induce primary human adipose progenitor cell death.** Human SC and OM adipose progenitor cells were grown to confluence. Cells were placed in serum-containing or serum free medium with 438 ng/mL of TNFα for 24 h or 48 h. Cell enumeration was performed on duplicate or triplicate dishes. Results are the mean+/-SE of 3 separate matched patient samples. *P<0.05, **P<0.01

## Results for Aim 2

### *MacCM on the Survival of Human Primary Adipose Progenitor Cells*

I determined whether there was a depot difference in the response to MacCM. Apoptosis was measured using Hoechst staining of condensed nuclei.

*Serum Deprivation on MacCM Survival Activity and Human Adipose Progenitor Cells*

MacCM did not suppress SC adipose progenitor cell apoptosis compared to the control media under conditions of serum withdrawal (Fig. 15). A similar 3.4 ± 0.2 % versus 3.5 ± 0.5 % apoptosis was observed with MacCM versus control media.

Under serum conditions, MacCM doubled the apoptosis of OM adipose progenitor cells versus the control media (3.1 ± 0.7 vs 1.7 ± 0.02) (Fig. 15), but the difference was not significant. MacCM suppressed OM adipose progenitor cell apoptosis compared to the control media under conditions of serum withdrawal. The mean apoptotic index was 6.6 ± 1.8 versus 7.8 ± 1.5 with MacCM versus control media, but the difference was not significant. Overall, there was significant OM adipose progenitor cell apoptosis in control media with serum deprivation ($P<0.01$), and a trend with MacCM but was not significant.

Serum withdrawal in control media resulted in a significant depot difference (SC, 3.49 ± 0.51%; OM, 7.82 ± 1.48%) ($P<0.05$) (Fig. 15). Serum deprivation in MacCM resulted in a depot difference but was not significant. This suggests that macrophages may have a pro-survival effect on OM adipose progenitor cells in the presence of serum withdrawal, resulting in an intrinsic depot difference. More studies will be needed to confirm this.

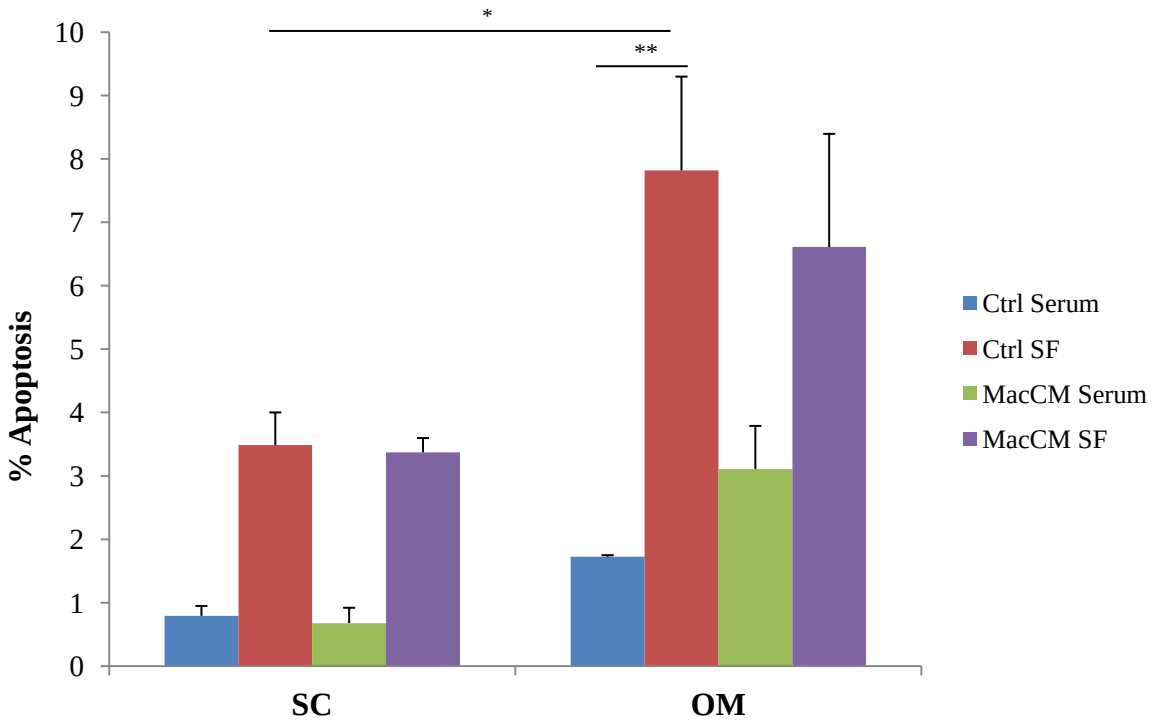


**Fig. 15 MacCM survival activity on primary human adipose progenitor cells in the presence of serum deprivation.** Human SC and OM progenitor cells were incubated for 6 h with serum-containing or serum-free medium in the presence of control medium or MacCM, as indicated. Apoptosis was assessed by quantification of Hoechst-stained apoptotic nuclei. N=3, *P<0.05, **P<0.001.

### *Serum Deprivation and TNFα on MacCM Survival Activity and Human Adipose Progenitor Cells*

MacCM did not suppress SC adipose progenitor cell apoptosis compared to the control media under conditions of serum withdrawal and TNFα (Fig. 16). A similar 2.3 ± 0.2 % versus 2.1 ± 0.1 % apoptosis was observed with MacCM versus control media.

Under serum conditions, MacCM doubled the apoptosis of OM adipose progenitor cells versus the control media (2.9 ± 0.5 vs 1.4 ± 0.3) (Fig. 16), but the difference was not significant. MacCM suppressed OM adipose progenitor cell apoptosis compared to the control media under conditions of serum withdrawal and TNFα. The mean apoptotic index was 4.7 ± 1.6 versus 6.4 ± 1.0 with MacCM versus control media, but the difference was not significant. Overall, there was significant OM adipose progenitor cell apoptosis in control media with serum deprivation and TNFα ($P<0.01$), and a trend with MacCM but was not significant.

Serum withdrawal and TNFα in control media resulted in a significant depot difference (SC, 2.1 ± 0.1%; OM, 6.4 ± 1%) ($P<0.01$) (Fig. 16). Serum deprivation and TNFα in MacCM resulted in a depot difference but was not significant. Therefore, we have observed for the first time that macrophages may have a pro-survival effect on OM adipose progenitor cells in the presence of serum deprivation and TNFα, resulting in an intrinsic depot difference.

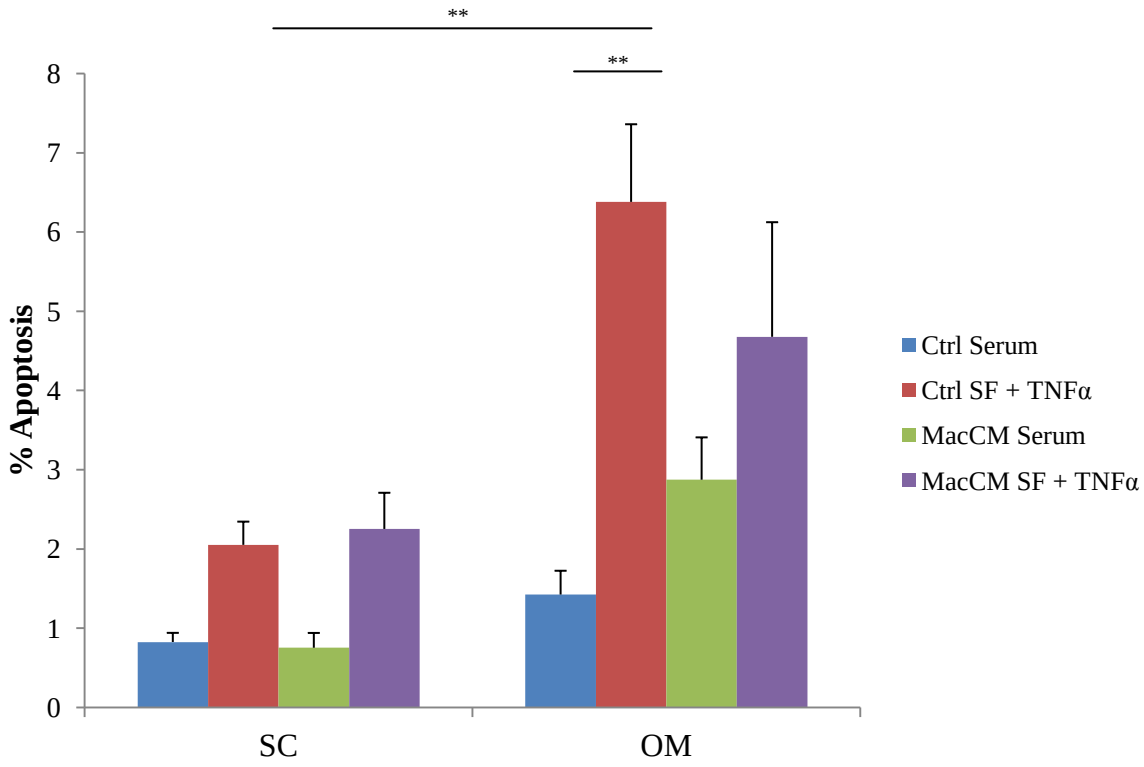


**Fig. 16 MacCM survival activity on primary human adipose progenitor cells in the presence of serum deprivation with TNFα.** Human SC and OM progenitor cells were incubated for 6 h with serum-containing or serum-free medium supplemented with 438 ng/ml TNFα in the presence of control medium or MacCM, as indicated. Apoptosis was assessed by quantification of Hoechst-stained apoptotic nuclei. N=4, **P<0.001.

### *Serum Deprivation and TNFα with CHX on MacCM Survival Activity and Human Adipose Progenitor Cells*

Serum deprivation with TNFα and CHX in MaCM and control media caused significant apoptosis in the SC depot ($P<0.01$). MacCM did not suppress SC adipose progenitor cell apoptosis compared to the control media under conditions of serum withdrawal and TNFα and CHX (Fig. 17). A similar 22.9 ± 5.8 % versus 24.6 ± 8.0 % apoptosis was observed with MacCM versus control medium.

Under serum conditions, MacCM doubled the apoptosis of OM adipose progenitor cells versus the control media (2.9 ± 0.5 vs 1.4 ± 0.3) (Fig. 17), but the difference was not significant. MacCM did not suppress OM adipose progenitor cell apoptosis compared to the control media under conditions of serum withdrawal and TNFα and CHX. The mean apoptotic index was 9.8 ± 0.6 versus 9.7 ± 1.2 with MacCM versus control media.

Serum withdrawal with TNFα and CHX in both MacCM and control media resulted in a trend of greater apoptosis in the SC depot compared to the OM depot but was not significant (Fig. 17). We have observed for the first time that that the SC depot showed more apoptosis than the OM depot. Macrophages did not appear to have a survival effect on either depot in the presence of serum deprivation with TNFα and CHX, eliminating any depot difference.

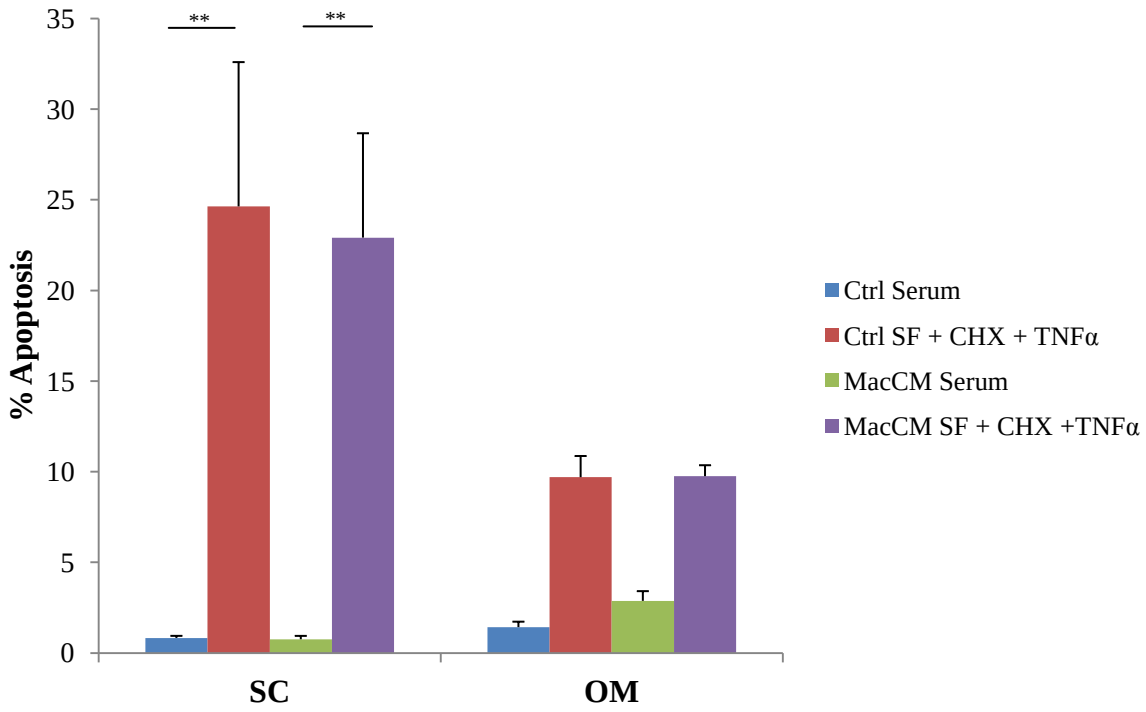


**Fig. 11 MacCM survival activity on primary human adipose progenitor cells in the presence of serum deprivation with TNFα and CHX.** Human subcutaneous and omental progenitor cells were incubated for 6h with serum-containing or serum-free medium supplemented with 10 mg/ml CHX and 10 ng/ml TNFα in the presence of control medium or MacCM, as indicated. Apoptosis was assessed by quantification of Hoechst-stained apoptotic nuclei. N=4, **P<0.001.

## 4.0 Discussion

Apoptosis is a cell death program that plays a crucial role in the regulation of tissue homeostasis (Elmore, 2007). Two main apoptotic pathways exist, the intrinsic (mitochondrial) pathway and the extrinsic (death receptor) pathway (Elmore, 2007; Fulda et al., 2010). The mitochondrial pathway is initiated by the release of apoptotic factors such as cytochrome c from the mitochondrial intermembrane space. The release of cytochrome c into the cytosol triggers caspase-3 activation through formation of the cytochrome c/Apaf-1/caspase-9-containing apoptosome complex. Stimulation of TNF death receptors, such as CD95 (APO-1/Fas), leads to the recruitment and activation of caspase-8 at the DISC, which in turn causes cleavage and activation of downstream effector caspases.

Few reports have been examined human adipose progenitor cell survival signaling. A recent report based on SGBS adipose progenitor cells implicates Akt2 in this response (Fischer-Posovszky et al., 2012). The reduced sensitivity of human adipose progenitor cells to apoptosis has been suggested to result from autocrine activation of pro-survival IGF-1 receptor signaling (Fischer-Posovszky et al., 2004).

My study included three pro-apoptotic conditions which involve distinct mechanisms for inducing apoptosis: serum withdrawal, serum withdrawal with TNFα and serum withdrawal with TNFα and CHX. We used two different cell death assays, Hoechst staining and cell enumeration, to examine depot-specific effects on adipose progenitor cell death.

Serum deprivation causes apoptosis by the removal of trophic factors essential for the survival of the cell (Kummer et al., 1997). Survival factor deprivation activates the intrinsic apoptotic pathway.

My study confirmed the greater predisposition of OM versus SC adipose progenitor cells to apoptosis when induced by serum deprivation. Hoechst staining revealed that serum deprivation led to significant apoptotic changes in 1.2% of SC versus 3.4% of OM adipose progenitor cells, respectively. Niesler et al was the first group to study apoptosis in human adipose progenitor cells from abdominal SC and OM depots (Niesler et al., 1998). They showed that serum deprivation led to apoptotic changes in 3% versus 7% of SC and OM adipose progenitor cells, respectively. They observed more overall apoptosis than me. Niesler et al. incubated their cells in serum-free media for 4 h and judged apoptosis by acridine orange staining of adherent cells. I deprived my cells of serum for 6 h and assessed apoptosis by Hoechst staining of adherent cells.

Cell enumeration data were consistent with Hoechst staining results, showing that OM versus SC adipose progenitor cells are more susceptible to cell death when induced by serum deprivation. Serum deprivation caused 19% versus 30% cell death in SC and OM adipose progenitor cells, respectively. Papineau et al. was the second group to publish a study that had examined depot-specific effects on adipose progenitor cell death, in which adipose progenitor cells derived from abdominal OM versus SC depots were found to be more susceptible to apoptosis (Papineau et al., 2003). They compared apoptosis of abdominal OM adipose progenitor cells with SC adipose progenitor cells subjected to serum deprivation. OM versus SC adipose progenitor cells exhibited a greater extent of percent cell death when placed in serum-free media (31% vs 16%). Papineau et al incubated their cells in serum-free media for 48 h, whereas my incubation time was a shorter 24 h. The depot difference could still be observed with a shorter duration of serum withdrawal.

I was able to demonstrate, with both of my cell death assays, that human primary adipose progenitor cells display a depot-specific susceptibility to cell death under conditions of serum withdrawal.

My study is the first to observe a positive correlation between BMI and cell death susceptibility between the two types of adipose progenitors (ratio OM/SC), when cell death is induced by serum deprivation. Further studies are needed to understand how BMI is linked to this difference in behavior.

Few reports have been published on depot-specific human adipose progenitor cell survival signaling. One study demonstrated that cIAP2 mRNA is expressed in a depot-specific manner (Niesler et al., 2001). cIAP2 is a potent inhibitor of apoptosis. In serum, cIAP2 mRNA was significantly over-expressed in OM versus SC adipose progenitor cells. Under conditions of serum withdrawal, cIAP2 mRNA expression was greater in SC versus OM adipose progenitor cells. This was due to a decrease in expression in the OM depot, whereas expression in the SC depot remained constant. In the presence of an apoptotic stimulus, lower cIAP2 expression in OM adipose progenitor cells may explain why they are more susceptible to death.

TNFα can induce cell death by activating caspase-8 or promote cell survival by activating NFκB (Gupta, 2002). TNFα induces apoptosis by binding to cell surface receptors CD95, TNFR1 (TNF Receptor-1), TNFR2 (TNF Receptor-2), TRAIL-R1 and TRAIL-R2 (Fraser and Evan, 1996; Fischer-Posovszky et al., 2004). Growth factor withdrawal and death receptor stimulation by TNFα activate both the extrinsic and intrinsic apoptotic pathways.

My study confirmed the larger predisposition of OM versus SC adipose progenitor cells to apoptosis when induced by serum deprivation and TNFα. Hoechst staining revealed that serum deprivation with TNFα led to significant apoptotic changes in 3.1% and 5.1% of SC and OM adipose progenitor cells, respectively. Niesler et al were the first group to study apoptosis induced by TNFα in human adipose progenitor cells from abdominal SC and OM depots (Niesler et al., 1998). They showed that serum deprivation with TNFα led to apoptotic changes in 4% versus 8.5% of SC and OM adipose progenitor cells, respectively. My study showed similar overall apoptosis to their study. Niesler et al incubated their cells in serum-free media with TNFα for 4 h and judged apoptosis by acridine orange staining of adherent cells. I deprived my cells of serum for 6 h and assessed apoptosis by Hoechst staining of adherent cells. Similarly to Niesler et al, we supplemented the serum-free media with 438 ng/mL of TNFα.

My study is the only one that has used cell enumeration to assess cell death induced by serum withdrawal in combination with TNFα. Cell enumeration revealed 28% and 40% cell death in of SC and OM adipose progenitor cells, respectively.

Both of my cell death assays showed that human primary adipose progenitor cells display a depot-specific susceptibility to cell death under conditions of serum withdrawal with TNFα.

I observed a negative correlation between cell death susceptibility between the two types of adipose progenitors (ratio OM/SC) and BMI, when cell death is induced by serum deprivation with TNFα. This was an unexpected result based on what I observed when cell death was induced with serum deprivation alone. It is unclear why stimulating the extrinsic cell death pathway would reverse the correlation. It appears that the addition of TNFα

dominates over the removal of trophic factors in terms of activating the cell death pathways. Further studies are necessary to examine whether BMI can influence TNFα receptor expression, since this could be one possible way that TNFa would act differently based on BMI.

CHX reduces levels of cellular FLIP, a negative regulator of apoptosis that acts as an endogenous brake on caspase-8 activation (Fischer-Posovszky et al., 2011; Fischer-Posovszky et al., 2004; Tchoukalova et al., 2007). CHX sensitizes human adipocytes to TNFα-induced apoptosis (Buchmann et al., 2007).

My study is the first to describe the effect of serum deprivation in combination with TNFα and CHX on OM versus SC adipose progenitor cells to apoptosis. The difference in the apoptotic response observed between SC versus OM adipose progenitor cells that I observed was lost when cell death is induced with the more potent combination of TNFα and CHX. Hoechst staining revealed that both SC and OM depots underwent significant apoptosis (14.8% vs 11.9%). The loss of a depot effect when cell death is induced with TNFα and CHX suggests that FLIP may play an important role in the depot-specific apoptotic susceptibility of adipose progenitor cells.

These apoptotic inducers have been used together by others (Fischer-Posovszky et al., 2011; Fischer-Posovszky et al., 2004; Molgat et al. 2012). Molgat et al observed an approximate 20% induction of human SC adipose progenitor cell apoptosis with this combination. Their study did not look at OM adipose progenitor cells. In both studies, the cells were incubated for 6 h with serum-free media supplemented with 10 mg/mL CHX and 10 ng/mL TNFα. Apoptosis was assessed by quantification of Hoechst-stained apoptotic nuclei.

Consistent with Hoechst staining, cell enumeration revealed no depot difference in percent cell death. Serum withdrawal in combination with TNFα and CHX caused 44% and 43% cell death in of SC and OM adipose progenitor cells, respectively. My data add information on this topic, since there are no other studies in the literature using cell enumeration to evaluate cell death in the different depots in response to TNFα and CHX.

The difference in the apoptotic response observed between SC versus OM adipose progenitor cells is lost when cell death is induced with the more potent combination of TNFα and CHX. The loss of a depot effect when cell death is induced with TNFα and CHX suggests that FLIP may play an important role in the depot-specific apoptotic susceptibility of adipose progenitor cells. In the setting of a strong TNFα signal, we can no longer discriminate between OM and SC depot cell death. The introduction of CHX maximizes the death signal, perhaps overwhelming the subtleties that may differentiate the responsiveness of the two depots.

Obesity is accompanied by the infiltration of circulating blood monocytes into AT, producing an abundant population of inflamed M1-type macrophages. This process of macrophage infiltration is associated with adipocyte hypertrophy (Bluher, 2010). Therefore, macrophages might influence adipose progenitor cells by altering their survival response. Studies on apoptosis using human macrophage models and human adipose progenitor cell models have been reported (Molgat et al., 2012). One study by Molgat et al discovered that J774A.1 and RAW264.7 MacCM inhibit 3T3-L1 adipose progenitor cell apoptosis induced by serum deprivation, and identified platelet-derived PDGF as the principal anti-apoptotic macrophage-secreted factor (Molgat et al., 2009). The identity of the factors from human macrophages mediating human adipocyte cell survival is not yet known. Interactions

between immune cells and adipose progenitor cells are important to consider because they may influence the number of adipose progenitor cells and/or their differentiation capacity and induce adipose tissue dysfunction by inhibiting overall adipogenic capacity.

My study is the first study to describe the survival activity of MacCM on SC versus OM adipose progenitor cells under the three pro-apoptotic conditions described.

Underconditions of serum withdrawal, Hoechst staining was used to assess apoptosis. MacCM did not have a survival effect on the SC depot. Furthermore, the OM depot did not show any significant changes in overall apoptosis, but some trends were seen. The OM depot showed more apoptosis in serum-containing MacCM compared to the control media. The extent of apoptosis with serum removal in MacCM appeared to be less than in control medium.

Under conditions of serum withdrawal and TNFα, Hoechst staining was used to assess apoptosis. The results are similar to what we observed with serum withdrawal alone. MacCM did not have a significant survival effect on the SC or OM depots. However, the OM depot showed more apoptosis in serum-containing MacCM compared to the control media. MacCM suppressed apoptosis in the OM depot with the removal of serum and addition of TNFα.

I examined survival activity of MacCM on SC and OM adipose progenitor cells under conditions of serum withdrawal in combination with TNFα and CHX. One other study has described the survival activity of MacCM under these conditions but solely for the SC depot. Molgat et al observed that MacCM is capable of preventing TNFα and CHX and serum deprivation-dependent SC adipose progenitor cell apoptosis; suggesting that it impedes intrinsic and extrinsic apoptotic pathways (Molgat et al., 2012). Hoechst staining

was used to assess apoptosis. Our results did not show a pro-survival effect of MacCM as seen by Molgat et al. The difference could be due to patient heterogeneity from the different donors. In my study, MacCM did not have a significant effect on either the SC or OM depot; however more overall apoptosis was seen in the SC depot. OM adipose progenitor cells appear to resist apoptosis better than SC adipose progenitor cells under these conditions, although these were trends that did not reach significance.

My study is mostly limited by donor variation effects on human adipose progenitor cells and partially on MD-macrophages. It should be noted that macrophages are highly influenced by the local environment in vivo, with different classes of cytokines resulting in pro-inflammatory (classic) versus anti-inflammatory (alternate) states of macrophage activation. Indeed, some investigators describe AT macrophages in intermediate states between these two extremes. I did not rigorously characterize my MacCM as pro- or anti-inflammatory. There may have been some variation in the activation state of the macrophages. Molgat et al observed that MacCM inhibited apoptosis of primary human adipose progenitor cells (Molgat et al., 2012). MacCM from IL4-treated anti-inflammatory macrophages, but not LPS-treated pro-inflammatory macrophages, was able to protect human SC adipose progenitor cells from apoptosis.

Future studies are needed to examine cell death and cell survival mechanisms in SC and OM adipose progenitor cells. Specifically, it would be beneficial to characterize TNFα receptors in adipose progenitors from the SC and OM depots. The positive BMI correlations, (with only 4 or 5 patients), gives impetus to perform larger analyses in future studies. It would also be important to understand how the activation state of macrophages influences depot susceptibility to apoptosis. Lastly, it would be useful to examine cellular proteins

involved in the pro-survival and pro-apoptotic pathways in both SC and OM adipose progenitors, such as members of the Bcl-2 family, to provide insight on the differences in depot-related death.

**Conclusion and Proposed Model**

Chronic positive energy imbalance leads to obesity. The survival of differentiation-competent adipose progenitor cells may influence the mechanism by which AT expands. Intrinsic (serum deprivation) and extrinsic (TNFα) apoptotic inducers cause more death in the OM versus SC depot (Fig. 18). When a more potent apoptotic stimulus (TNFα and CHX) is used, the depot difference is lost. Since OM adipose progenitor cells have a greater predisposition to cell death, under mild apoptotic conditions, AT from the OM depot may expand via hypertrophy in patients with higher BMIs. The *in vitro* adipose progenitor cell survival function of macrophage-secreted factors raises the possibility that ATMs may function to promote adipose progenitor cell survival *in vivo*, which may be depot dependent. I looked at the effect of macrophages under the same three apoptotic conditions. I did not observe a macrophage effect in either depot. My study did not support previous work which suggested a pro-survival macrophage effect on the SC depot. Reasons for the observed differences are not clear, but donor heterogeneity may be a factor.

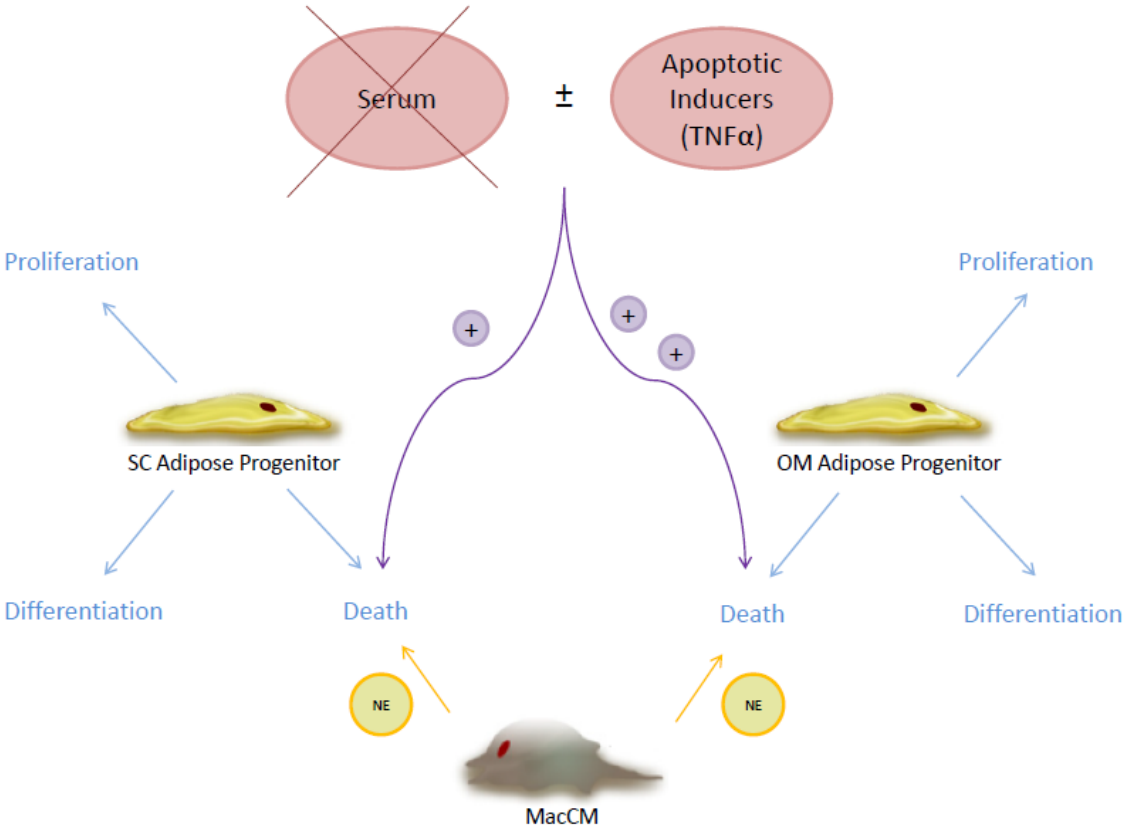


**Fig. 18 Model of how apoptotic inducers, either intrinsic (serum withdrawal) or extrinsic (TNFα), influence the apoptotic susceptibility of adipose progenitor cells.** A depot-dependent property was observed, with a higher degree of apoptotic sensitivity to serum withdrawal or serum withdrawal in combination with TNFα in OM versus SC abdominal human adipose progenitor cells. MacCM obtained from unactivated macrophages showed no effect on adipose progenitor cell survival in the presence of two apoptotic stimuli.

## 5.0 References


Ahmadian, M., Wang, Y., and Sul, H.S. (2010). Lipolysis in adipocytes. Int. J. Biochem. Cell Biol. *42,* 555-559.

Alkhouri, N., Gornicka, A., Berk, M.P., Thapaliya, S., Dixon, L.J., Kashyap, S., Schauer, P.R., and Feldstein, A.E. (2010). Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis. J. Biol. Chem. *285,* 3428-3438.

Arner, E., Westermark, P.O., Spalding, K.L., Britton, T., Ryden, M., Frisen, J., Bernard, S., and Arner, P. (2010). Adipocyte turnover: relevance to human adipose tissue morphology. Diabetes *59,* 105-109.

Arner, P., and Spalding, K.L. (2010). Fat cell turnover in humans. Biochem. Biophys. Res. Commun. *396,* 101-104.

Artemenko, Y., Gagnon, A., Aubin, D., and Sorisky, A. (2005). Anti-adipogenic effect of PDGF is reversed by PKC inhibition. J. Cell. Physiol. *204,* 646-653.

Ashkenazi, A., and Dixit, V.M. (1998). Death receptors: signaling and modulation. Science *281,* 1305-1308.

Bergsbaken, T., Fink, S.L., and Cookson, B.T. (2009). Pyroptosis: host cell death and inflammation. Nat. Rev. Microbiol. *7,* 99-109.

Bjorbaek, C., and Kahn, B.B. (2004). Leptin signaling in the central nervous system and the periphery. Recent Prog. Horm. Res. *59,* 305-331.

Bluher, M. (2010). The distinction of metabolically 'healthy' from 'unhealthy' obese individuals. Curr. Opin. Lipidol. *21,* 38-43.

Boden, G. (2009). Endoplasmic reticulum stress: another link between obesity and insulin resistance/inflammation? Diabetes *58,* 518-519.

Bradley, R.L., Fisher, F.F., and Maratos-Flier, E. (2008). Dietary fatty acids differentially regulate production of TNF-alpha and IL-10 by murine 3T3-L1 adipocytes. Obesity (Silver Spring) *16,* 938-944.

Bratton, D.L., Fadok, V.A., Richter, D.A., Kailey, J.M., Guthrie, L.A., and Henson, P.M. (1997). Appearance of phosphatidylserine on apoptotic cells requires calcium-mediated nonspecific flip-flop and is enhanced by loss of the aminophospholipid translocase. J. Biol. Chem. *272,* 26159-26165.

Buchmann, J., Meyer, C., Neschen, S., Augustin, R., Schmolz, K., Kluge, R., Al-Hasani, H., Jurgens, H., Eulenberg, K., Wehr, R., *et al.* (2007). Ablation of the cholesterol transporter

adenosine triphosphate-binding cassette transporter G1 reduces adipose cell size and protects against diet-induced obesity. Endocrinology *148*, 1561-1573.

Cai, J., Yang, J., and Jones, D.P. (1998). Mitochondrial control of apoptosis: the role of cytochrome c. Biochim. Biophys. Acta *1366,* 139-149.

Calder, P.C., Ahluwalia, N., Brouns, F., Buetler, T., Clement, K., Cunningham, K., Esposito, K., Jonsson, L.S., Kolb, H., Lansink, M., *et al.* (2011). Dietary factors and low-grade inflammation in relation to overweight and obesity. Br. J. Nutr. *106 Suppl 3*, S5-78.

Calton, M.A., Ersoy, B.A., Zhang, S., Kane, J.P., Malloy, M.J., Pullinger, C.R., Bromberg, Y., Pennacchio, L.A., Dent, R., McPherson, R., Ahituv, N., and Vaisse, C. (2009). Association of functionally significant Melanocortin-4 but not Melanocortin-3 receptor mutations with severe adult obesity in a large North American case-control study. Hum. Mol. Genet. *18,* 1140-1147.

Cannon, B., Hedin, A., and Nedergaard, J. (1982). Exclusive occurrence of thermogenin antigen in brown adipose tissue. FEBS Lett. *150,* 129-132.

Cawthorn, W.P., Scheller, E.L., and MacDougald, O.A. (2012). Adipose tissue stem cells meet preadipocyte commitment: going back to the future. J. Lipid Res. *53,* 227-246.

Chau, Y.Y., Bandiera, R., Serrels, A., Martinez-Estrada, O.M., Qing, W., Lee, M., Slight, J., Thornburn, A., Berry, R., McHaffie, S., *et al.* (2014). Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. Nat. Cell Biol. *16,* 367-375.

Chicheportiche, Y., Bourdon, P.R., Xu, H., Hsu, Y.M., Scott, H., Hession, C., Garcia, I., and Browning, J.L. (1997). TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J. Biol. Chem. *272,* 32401-32410.

Chinnaiyan, A.M. (1999). The apoptosome: heart and soul of the cell death machine. Neoplasia *1,* 5-15.

Cinti, S., Mitchell, G., Barbatelli, G., Murano, I., Ceresi, E., Faloia, E., Wang, S., Fortier, M., Greenberg, A.S., and Obin, M.S. (2005). Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J. Lipid Res. *46,* 2347-2355.

Collins, D., Hogan, A.M., O'Shea, D., and Winter, D.C. (2009). The omentum: anatomical, metabolic, and surgical aspects. J. Gastrointest. Surg. *13,* 1138-1146.

Constant, V.A., Gagnon, A., Landry, A., and Sorisky, A. (2006). Macrophage-conditioned medium inhibits the differentiation of 3T3-L1 and human abdominal preadipocytes. Diabetologia *49,* 1402-1411.

Conway, B., and Rene, A. (2004). Obesity as a disease: no lightweight matter. Obes. Rev. *5,* 145-151.

Cory, S., and Adams, J.M. (2002). The Bcl2 family: regulators of the cellular life-or-death switch. Nat. Rev. Cancer. *2,* 647-656.

Cypess, A.M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A.B., Kuo, F.C., Palmer, E.L., Tseng, Y.H., Doria, A., Kolodny, G.M., and Kahn, C.R. (2009). Identification and importance of brown adipose tissue in adult humans. N. Engl. J. Med. *360,* 1509-1517.

Daigneault, M., Preston, J.A., Marriott, H.M., Whyte, M.K., and Dockrell, D.H. (2010). The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages. PLoS One *5,* e8668.

Dalmas, E., Clement, K., and Guerre-Millo, M. (2011). Defining macrophage phenotype and function in adipose tissue. Trends Immunol. *32,* 307-314.

Danforth, E.,Jr. (2000). Failure of adipocyte differentiation causes type II diabetes mellitus? Nat. Genet. *26,* 13.

de Ferranti, S., and Mozaffarian, D. (2008). The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. Clin. Chem. *54,* 945-955.

Decaunes, P., Esteve, D., Zakaroff-Girard, A., Sengenes, C., Galitzky, J., and Bouloumie, A. (2011). Adipose-derived stromal cells: cytokine expression and immune cell contaminants. Methods Mol. Biol. *702,* 151-161.

Du, C., Fang, M., Li, Y., Li, L., and Wang, X. (2000). Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell *102,* 33-42.

Duffaut, C., Galitzky, J., Lafontan, M., and Bouloumie, A. (2009). Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. Biochem. Biophys. Res. Commun. *384,* 482-485.

Elmore, S. (2007). Apoptosis: a review of programmed cell death. Toxicol. Pathol. *35,* 495-516.

Farooqi, I.S., Matarese, G., Lord, G.M., Keogh, J.M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S.A., Perna, F., Fontana, S., *et al.* (2002). Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J. Clin. Invest. *110,* 1093-1103.

Feng, D., Tang, Y., Kwon, H., Zong, H., Hawkins, M., Kitsis, R.N., and Pessin, J.E. (2011). High-fat diet-induced adipocyte cell death occurs through a cyclophilin D intrinsic signaling pathway independent of adipose tissue inflammation. Diabetes *60,* 2134-2143.

Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A.B., Benoist, C., Shoelson, S., and Mathis, D. (2009). Lean, but not obese, fat is enriched

for a unique population of regulatory T cells that affect metabolic parameters. Nat. Med. *15,* 930-939.

Fink, S.L., and Cookson, B.T. (2005). Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect. Immun. *73,* 1907-1916.

Fischer-Posovszky, P., Keuper, M., Nagel, S., Hesse, D., Schurmann, A., Debatin, K.M., Strauss, G., and Wabitsch, M. (2011). Downregulation of FLIP by cycloheximide sensitizes human fat cells to CD95-induced apoptosis. Exp. Cell Res. *317,* 2200-2209.

Fischer-Posovszky, P., Tews, D., Horenburg, S., Debatin, K.M., and Wabitsch, M. (2012). Differential function of Akt1 and Akt2 in human adipocytes. Mol. Cell. Endocrinol. *358,* 135-143.

Fischer-Posovszky, P., Tornqvist, H., Debatin, K.M., and Wabitsch, M. (2004). Inhibition of death-receptor mediated apoptosis in human adipocytes by the insulin-like growth factor I (IGF-I)/IGF-I receptor autocrine circuit. Endocrinology *145,* 1849-1859.

Fischer-Posovszky, P., Wang, Q.A., Asterholm, I.W., Rutkowski, J.M., and Scherer, P.E. (2011). Targeted deletion of adipocytes by apoptosis leads to adipose tissue recruitment of alternatively activated M2 macrophages. Endocrinology *152,* 3074-3081.

Fontana, L., Eagon, J.C., Trujillo, M.E., Scherer, P.E., and Klein, S. (2007). Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes *56,* 1010-1013.

Franco, M., Martinez, F., Rodriguez-Iturbe, B., Johnson, R.J., Santamaria, J., Montoya, A., Nepomuceno, T., Bautista, R., Tapia, E., and Herrera-Acosta, J. (2006). Angiotensin II, interstitial inflammation, and the pathogenesis of salt-sensitive hypertension. Am. J. Physiol. Renal Physiol. *291,* F1281-7.

Fraser, A., and Evan, G. (1996). A license to kill. Cell *85,* 781-784.

Frayling, T.M., Timpson, N.J., Weedon, M.N., Zeggini, E., Freathy, R.M., Lindgren, C.M., Perry, J.R., Elliott, K.S., Lango, H., Rayner, N.W., *et al.* (2007). A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science *316,* 889-894.

Frayn, K.N., Karpe, F., Fielding, B.A., Macdonald, I.A., and Coppack, S.W. (2003). Integrative physiology of human adipose tissue. Int. J. Obes. Relat. Metab. Disord. *27,* 875-888.

Fulda, S., Gorman, A.M., Hori, O., and Samali, A. (2010). Cellular stress responses: cell survival and cell death. Int. J. Cell. Biol. *2010,* 214074.

Gealekman, O., Guseva, N., Hartigan, C., Apotheker, S., Gorgoglione, M., Gurav, K., Tran, K.V., Straubhaar, J., Nicoloro, S., Czech, M.P., *et al.* (2011). Depot-specific differences and insufficient subcutaneous adipose tissue angiogenesis in human obesity. Circulation *123,* 186-194.

Gesta, S., Tseng, Y.H., and Kahn, C.R. (2007). Developmental origin of fat: tracking obesity to its source. Cell *131,* 242-256.

Grimm, S., Stanger, B.Z., and Leder, P. (1996). RIP and FADD: two "death domain"-containing proteins can induce apoptosis by convergent, but dissociable, pathways. Proc. Natl. Acad. Sci. U. S. A. *93,* 10923-10927.

Gupta, S. (2002). A decision between life and death during TNF-alpha-induced signaling. J. Clin. Immunol. *22,* 185-194.

Hacker, G. (2000). The morphology of apoptosis. Cell Tissue Res. *301,* 5-17.

Hainerova, I., Larsen, L.H., Holst, B., Finkova, M., Hainer, V., Lebl, J., Hansen, T., and Pedersen, O. (2007). Melanocortin 4 receptor mutations in obese Czech children: studies of prevalence, phenotype development, weight reduction response, and functional analysis. J. Clin. Endocrinol. Metab. *92,* 3689-3696.

Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., and Friedman, J.M. (1995). Weight-reducing effects of the plasma protein encoded by the obese gene. Science *269,* 543-546.

Han, T.S., Sattar, N., and Lean, M. (2006). ABC of obesity. Assessment of obesity and its clinical implications. Bmj *333,* 695-698.

Harms, M., and Seale, P. (2013). Brown and beige fat: development, function and therapeutic potential. Nat. Med. *19,* 1252-1263.

Hauner, H. (2005). Secretory factors from human adipose tissue and their functional role. Proc. Nutr. Soc. *64,* 163-169.

Hayashi, H., Campenot, R.B., Vance, D.E., and Vance, J.E. (2007). Apolipoprotein E-containing lipoproteins protect neurons from apoptosis via a signaling pathway involving low-density lipoprotein receptor-related protein-1. J. Neurosci. *27,* 1933-1941.

Heilbronn, L., Smith, S.R., and Ravussin, E. (2004). Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. Int. J. Obes. Relat. Metab. Disord. *28 Suppl 4,* S12-21.

Hill, M.M., Adrain, C., Duriez, P.J., Creagh, E.M., and Martin, S.J. (2004). Analysis of the composition, assembly kinetics and activity of native Apaf-1 apoptosomes. Embo j. *23,* 2134-2145.

Hinney, A., Bettecken, T., Tarnow, P., Brumm, H., Reichwald, K., Lichtner, P., Scherag, A., Nguyen, T.T., Schlumberger, P., Rief, W., *et al.* (2006). Prevalence, spectrum, and functional characterization of melanocortin-4 receptor gene mutations in a representative population-based sample and obese adults from Germany. J. Clin. Endocrinol. Metab. *91,* 1761-1769.

Hinnouho, G.M., Czernichow, S., Dugravot, A., Batty, G.D., Kivimaki, M., and Singh-Manoux, A. (2013). Metabolically healthy obesity and risk of mortality: does the definition of metabolic health matter? Diabetes Care *36,* 2294-2300.

Hirosumi, J., Tuncman, G., Chang, L., Gorgun, C.Z., Uysal, K.T., Maeda, K., Karin, M., and Hotamisligil, G.S. (2002). A central role for JNK in obesity and insulin resistance. Nature *420,* 333-336.

Hotamisligil, G.S., Arner, P., Caro, J.F., Atkinson, R.L., and Spiegelman, B.M. (1995). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J. Clin. Invest. *95,* 2409-2415.

Hotamisligil, G.S., and Erbay, E. (2008). Nutrient sensing and inflammation in metabolic diseases. Nat. Rev. Immunol. *8,* 923-934.

Hotamisligil, G.S., Shargill, N.S., and Spiegelman, B.M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science *259,* 87-91.

Hsu, H., Xiong, J., and Goeddel, D.V. (1995). The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. Cell *81,* 495-504.

Ibrahim, M.M. (2010). Subcutaneous and visceral adipose tissue: structural and functional differences. Obes. Rev. *11,* 11-18.

Igney, F.H., and Krammer, P.H. (2002). Death and anti-death: tumour resistance to apoptosis. Nat. Rev. Cancer. *2,* 277-288.

Jiang, C., Qu, A., Matsubara, T., Chanturiya, T., Jou, W., Gavrilova, O., Shah, Y.M., and Gonzalez, F.J. (2011). Disruption of hypoxia-inducible factor 1 in adipocytes improves insulin sensitivity and decreases adiposity in high-fat diet-fed mice. Diabetes *60,* 2484-2495.

Jiao, P., Ma, J., Feng, B., Zhang, H., Diehl, J.A., Chin, Y.E., Yan, W., and Xu, H. (2011). FFA-induced adipocyte inflammation and insulin resistance: involvement of ER stress and IKKbeta pathways. Obesity (Silver Spring) *19,* 483-491.

Johnson, W.D.,Jr, Mei, B., and Cohn, Z.A. (1977). The separation, long-term cultivation, and maturation of the human monocyte. J. Exp. Med. *146,* 1613-1626.

Karelis, A.D. (2008). Metabolically healthy but obese individuals. Lancet *372,* 1281-1283.

Karelis, A.D., Brochu, M., and Rabasa-Lhoret, R. (2004). Can we identify metabolically healthy but obese individuals (MHO)? Diabetes Metab. *30,* 569-572.

Kataoka, T., Schroter, M., Hahne, M., Schneider, P., Irmler, M., Thome, M., Froelich, C.J., and Tschopp, J. (1998). FLIP prevents apoptosis induced by death receptors but not by perforin/granzyme B, chemotherapeutic drugs, and gamma irradiation. J. Immunol. *161,* 3936-3942.

Keophiphath, M., Achard, V., Henegar, C., Rouault, C., Clement, K., and Lacasa, D. (2009). Macrophage-secreted factors promote a profibrotic phenotype in human preadipocytes. Mol. Endocrinol. *23,* 11-24.

Kerr, J.F., Wyllie, A.H., and Currie, A.R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer *26,* 239-257.

Keuper, M., Bluher, M., Schon, M.R., Moller, P., Dzyakanchuk, A., Amrein, K., Debatin, K.M., Wabitsch, M., and Fischer-Posovszky, P. (2011). An inflammatory micro-environment promotes human adipocyte apoptosis. Mol. Cell. Endocrinol. *339,* 105-113.

Khandekar, M.J., Cohen, P., and Spiegelman, B.M. (2011). Molecular mechanisms of cancer development in obesity. Nat. Rev. Cancer. *11,* 886-895.

Kischkel, F.C., Hellbardt, S., Behrmann, I., Germer, M., Pawlita, M., Krammer, P.H., and Peter, M.E. (1995). Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. Embo j. *14,* 5579-5588.

Kloting, N., Fasshauer, M., Dietrich, A., Kovacs, P., Schon, M.R., Kern, M., Stumvoll, M., and Bluher, M. (2010). Insulin-sensitive obesity. Am. J. Physiol. Endocrinol. Metab. *299,* E506-15.

Knowles, K.M., Paiva, L.L., Sanchez, S.E., Revilla, L., Lopez, T., Yasuda, M.B., Yanez, N.D., Gelaye, B., and Williams, M.A. (2011). Waist Circumference, Body Mass Index, and Other Measures of Adiposity in Predicting Cardiovascular Disease Risk Factors among Peruvian Adults. Int. J. Hypertens. *2011,* 931402.

Krakoff, J., Ma, L., Kobes, S., Knowler, W.C., Hanson, R.L., Bogardus, C., and Baier, L.J. (2008). Lower metabolic rate in individuals heterozygous for either a frameshift or a functional missense MC4R variant. Diabetes *57,* 3267-3272.

Kummer, J.L., Rao, P.K., and Heidenreich, K.A. (1997). Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. J. Biol. Chem. *272,* 20490-20494.

Kurosaka, K., Takahashi, M., Watanabe, N., and Kobayashi, Y. (2003). Silent cleanup of very early apoptotic cells by macrophages. J. Immunol. *171,* 4672-4679.

Lacasa, D., Taleb, S., Keophiphath, M., Miranville, A., and Clement, K. (2007). Macrophage-secreted factors impair human adipogenesis: involvement of proinflammatory state in preadipocytes. Endocrinology *148,* 868-877.

Lago, F., Dieguez, C., Gomez-Reino, J., and Gualillo, O. (2007). Adipokines as emerging mediators of immune response and inflammation. Nat. Clin. Pract. Rheumatol. *3,* 716-724.

Lago, F., Dieguez, C., Gomez-Reino, J., and Gualillo, O. (2007). The emerging role of adipokines as mediators of inflammation and immune responses. Cytokine Growth Factor Rev. *18,* 313-325.

Lau, D.C., Dhillon, B., Yan, H., Szmitko, P.E., and Verma, S. (2005). Adipokines: molecular links between obesity and atheroslcerosis. Am. J. Physiol. Heart Circ. Physiol. *288,* H2031-41.

Linkermann, A., and Green, D.R. (2014). Necroptosis. N. Engl. J. Med. *370,* 455-465.

Locksley, R.M., Killeen, N., and Lenardo, M.J. (2001). The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell *104,* 487-501.

Lubrano-Berthelier, C., Dubern, B., Lacorte, J.M., Picard, F., Shapiro, A., Zhang, S., Bertrais, S., Hercberg, S., Basdevant, A., Clement, K., and Vaisse, C. (2006). Melanocortin 4 receptor mutations in a large cohort of severely obese adults: prevalence, functional classification, genotype-phenotype relationship, and lack of association with binge eating. J. Clin. Endocrinol. Metab. *91,* 1811-1818.

Macotela, Y., Emanuelli, B., Mori, M.A., Gesta, S., Schulz, T.J., Tseng, Y.H., and Kahn, C.R. (2012). Intrinsic differences in adipocyte precursor cells from different white fat depots. Diabetes *61,* 1691-1699.

Magun, R., Boone, D.L., Tsang, B.K., and Sorisky, A. (1998). The effect of adipocyte differentiation on the capacity of 3T3-L1 cells to undergo apoptosis in response to growth factor deprivation. Int. J. Obes. Relat. Metab. Disord. *22,* 567-571.

Makki, K., Froguel, P., and Wolowczuk, I. (2013). Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inflamm. *2013,* 139239.

Manninen, A.H. (2006). Very-low-carbohydrate diets and preservation of muscle mass. Nutr. Metab. (Lond) *3,* 9.

Martinez, F.O., Gordon, S., Locati, M., and Mantovani, A. (2006). Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J. Immunol. *177,* 7303-7311.

Maumus, M., Sengenes, C., Decaunes, P., Zakaroff-Girard, A., Bourlier, V., Lafontan, M., Galitzky, J., and Bouloumie, A. (2008). Evidence of in situ proliferation of adult adipose tissue-derived progenitor cells: influence of fat mass microenvironment and growth. J. Clin. Endocrinol. Metab. *93,* 4098-4106.

Mayi, T.H., Duhem, C., Copin, C., Bouhlel, M.A., Rigamonti, E., Pattou, F., Staels, B., and Chinetti-Gbaguidi, G. (2010). Visfatin is induced by peroxisome proliferator-activated receptor gamma in human macrophages. Febs j. *277,* 3308-3320.

McCarthy, M.I. (2010). Genomics, type 2 diabetes, and obesity. N. Engl. J. Med. *363,* 2339-2350.

McDonnell, M.E., Ganley-Leal, L.M., Mehta, A., Bigornia, S.J., Mott, M., Rehman, Q., Farb, M.G., Hess, D.T., Joseph, L., Gokce, N., and Apovian, C.M. (2012). B lymphocytes in human subcutaneous adipose crown-like structures. Obesity (Silver Spring) *20,* 1372-1378.

McGuire, S. (2011). Shields M., Carroll M.D., Ogden C.L. adult obesity prevalence in Canada and the United States. NCHS data brief no. 56, Hyattsville, MD: National Center for Health Statistics, 2011. Adv. Nutr. *2,* 368-369.

McNelis, J.C., and Olefsky, J.M. (2014). Macrophages, Immunity, and Metabolic Disease. Immunity *41,* 36-48.

Messier, V., Karelis, A.D., Prud'homme, D., Primeau, V., Brochu, M., and Rabasa-Lhoret, R. (2010). Identifying metabolically healthy but obese individuals in sedentary postmenopausal women. Obesity (Silver Spring) *18,* 911-917.

Molgat, A.S., Gagnon, A., Foster, C., and Sorisky, A. (2012). The activation state of macrophages alters their ability to suppress preadipocyte apoptosis. J. Endocrinol. *214,* 21-29.

Molgat, A.S., Gagnon, A., and Sorisky, A. (2011). Macrophage-induced preadipocyte survival depends on signaling through Akt, ERK1/2, and reactive oxygen species. Exp. Cell Res. *317,* 521-530.

Molgat, A.S., Gagnon, A., and Sorisky, A. (2009). Preadipocyte apoptosis is prevented by macrophage-conditioned medium in a PDGF-dependent manner. Am. J. Physiol. Cell. Physiol. *296,* C757-65.

Montague, C.T., Farooqi, I.S., Whitehead, J.P., Soos, M.A., Rau, H., Wareham, N.J., Sewter, C.P., Digby, J.E., Mohammed, S.N., Hurst, J.A., *et al.* (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature *387,* 903-908.

Mora, S., and Pessin, J.E. (2002). An adipocentric view of signaling and intracellular trafficking. Diabetes Metab. Res. Rev. *18,* 345-356.

Morgan, S.C., Taylor, D.L., and Pocock, J.M. (2004). Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. J. Neurochem. *90,* 89-101.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C., Abera, S.F., *et al.* (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet

Niesler, C.U., Prins, J.B., O'Rahilly, S., Siddle, K., and Montague, C.T. (2001). Adipose depot-specific expression of cIAP2 in human preadipocytes and modulation of expression by serum factors and TNFalpha. Int. J. Obes. Relat. Metab. Disord. *25,* 1027-1033.

Niesler, C.U., Siddle, K., and Prins, J.B. (1998). Human preadipocytes display a depot-specific susceptibility to apoptosis. Diabetes *47,* 1365-1368.

Niesler, C.U., Urso, B., Prins, J.B., and Siddle, K. (2000). IGF-I inhibits apoptosis induced by serum withdrawal, but potentiates TNF-alpha-induced apoptosis, in 3T3-L1 preadipocytes. J. Endocrinol. *167,* 165-174.

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., *et al.* (2009). CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat. Med. *15,* 914-920.

Onate, B., Vilahur, G., Ferrer-Lorente, R., Ybarra, J., Diez-Caballero, A., Ballesta-Lopez, C., Moscatiello, F., Herrero, J., and Badimon, L. (2012). The subcutaneous adipose tissue reservoir of functionally active stem cells is reduced in obese patients. Faseb j. *26,* 4327-4336.

Osborn, O., and Olefsky, J.M. (2012). The cellular and signaling networks linking the immune system and metabolism in disease. Nat. Med. *18,* 363-374.

Ouchi, N., Higuchi, A., Ohashi, K., Oshima, Y., Gokce, N., Shibata, R., Akasaki, Y., Shimono, A., and Walsh, K. (2010). Sfrp5 is an anti-inflammatory adipokine that modulates metabolic dysfunction in obesity. Science *329,* 454-457.

Ouellet, V., Labbe, S.M., Blondin, D.P., Phoenix, S., Guerin, B., Haman, F., Turcotte, E.E., Richard, D., and Carpentier, A.C. (2012). Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J. Clin. Invest. *122,* 545-552.

Ozcan, U., Cao, Q., Yilmaz, E., Lee, A.H., Iwakoshi, N.N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L.H., and Hotamisligil, G.S. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science *306,* 457-461.

Ozcan, U., Yilmaz, E., Ozcan, L., Furuhashi, M., Vaillancourt, E., Smith, R.O., Gorgun, C.Z., and Hotamisligil, G.S. (2006). Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science *313,* 1137-1140.

Papineau, D., Gagnon, A., and Sorisky, A. (2003). Apoptosis of human abdominal preadipocytes before and after differentiation into adipocytes in culture. Metabolism *52,* 987-992.

Peter, M.E., and Krammer, P.H. (1998). Mechanisms of CD95 (APO-1/Fas)-mediated apoptosis. Curr. Opin. Immunol. *10,* 545-551.

Primeau, V., Coderre, L., Karelis, A.D., Brochu, M., Lavoie, M.E., Messier, V., Sladek, R., and Rabasa-Lhoret, R. (2011). Characterizing the profile of obese patients who are metabolically healthy. Int. J. Obes. (Lond) *35,* 971-981.

Prins, J.B., Niesler, C.U., Winterford, C.M., Bright, N.A., Siddle, K., O'Rahilly, S., Walker, N.I., and Cameron, D.P. (1997). Tumor necrosis factor-alpha induces apoptosis of human adipose cells. Diabetes *46,* 1939-1944.

Ramachandrappa, S., and Farooqi, I.S. (2011). Genetic approaches to understanding human obesity. J. Clin. Invest. *121,* 2080-2086.

Rasmussen, M.S., Lihn, A.S., Pedersen, S.B., Bruun, J.M., Rasmussen, M., and Richelsen, B. (2006). Adiponectin receptors in human adipose tissue: effects of obesity, weight loss, and fat depots. Obesity (Silver Spring) *14,* 28-35.

Rausch, M.E., Weisberg, S., Vardhana, P., and Tortoriello, D.V. (2008). Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. Int. J. Obes. (Lond) *32,* 451-463.

Rodeheffer, M.S., Birsoy, K., and Friedman, J.M. (2008). Identification of white adipocyte progenitor cells in vivo. Cell *135,* 240-249.

Romeo, G.R., Lee, J., and Shoelson, S.E. (2012). Metabolic syndrome, insulin resistance, and roles of inflammation--mechanisms and therapeutic targets. Arterioscler. Thromb. Vasc. Biol. *32,* 1771-1776.

Rubio-Moscardo, F., Blesa, D., Mestre, C., Siebert, R., Balasas, T., Benito, A., Rosenwald, A., Climent, J., Martinez, J.I., Schilhabel, M., *et al.* (2005). Characterization of 8p21.3 chromosomal deletions in B-cell lymphoma: TRAIL-R1 and TRAIL-R2 as candidate dosage-dependent tumor suppressor genes. Blood *106,* 3214-3222.

Saelens, X., Festjens, N., Vande Walle, L., van Gurp, M., van Loo, G., and Vandenabeele, P. (2004). Toxic proteins released from mitochondria in cell death. Oncogene *23,* 2861-2874.

Sakahira, H., Enari, M., and Nagata, S. (1998). Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature *391,* 96-99.

Savill, J., and Fadok, V. (2000). Corpse clearance defines the meaning of cell death. Nature *407,* 784-788.

Scaffidi, C., Schmitz, I., Krammer, P.H., and Peter, M.E. (1999). The role of c-FLIP in modulation of CD95-induced apoptosis. J. Biol. Chem. *274,* 1541-1548.

Schimmer, A.D. (2004). Inhibitor of apoptosis proteins: translating basic knowledge into clinical practice. Cancer Res. *64,* 7183-7190.

Seale, P., Conroe, H.M., Estall, J., Kajimura, S., Frontini, A., Ishibashi, J., Cohen, P., Cinti, S., and Spiegelman, B.M. (2011). Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J. Clin. Invest. *121,* 96-105.

Shoelson, S.E., Lee, J., and Goldfine, A.B. (2006). Inflammation and insulin resistance. J. Clin. Invest. *116,* 1793-1801.

Skurk, T., and Hauner, H. (2012). Primary culture of human adipocyte precursor cells: expansion and differentiation. Methods Mol. Biol. *806,* 215-226.

Slee, E.A., Adrain, C., and Martin, S.J. (2001). Executioner caspase-3, -6, and -7 perform distinct, non-redundant roles during the demolition phase of apoptosis. J. Biol. Chem. *276,* 7320-7326.

Smemo, S., Tena, J.J., Kim, K.H., Gamazon, E.R., Sakabe, N.J., Gomez-Marin, C., Aneas, I., Credidio, F.L., Sobreira, D.R., Wasserman, N.F., *et al.* (2014). Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature *507,* 371-375.

Sorisky, A., Molgat, A.S., and Gagnon, A. (2013). Macrophage-induced adipose tissue dysfunction and the preadipocyte: should I stay (and differentiate) or should I go? Adv. Nutr. *4,* 67-75.

Spalding, K.L., Arner, E., Westermark, P.O., Bernard, S., Buchholz, B.A., Bergmann, O., Blomqvist, L., Hoffstedt, J., Naslund, E., Britton, T., *et al.* (2008). Dynamics of fat cell turnover in humans. Nature *453,* 783-787.

Stienstra, R., Duval, C., Keshtkar, S., van der Laak, J., Kersten, S., and Muller, M. (2008). Peroxisome proliferator-activated receptor gamma activation promotes infiltration of alternatively activated macrophages into adipose tissue. J. Biol. Chem. *283,* 22620-22627.

Strissel, K.J., Stancheva, Z., Miyoshi, H., Perfield, J.W.,2nd, DeFuria, J., Jick, Z., Greenberg, A.S., and Obin, M.S. (2007). Adipocyte death, adipose tissue remodeling, and obesity complications. Diabetes *56,* 2910-2918.

Stutzmann, F., Tan, K., Vatin, V., Dina, C., Jouret, B., Tichet, J., Balkau, B., Potoczna, N., Horber, F., O'Rahilly, S., *et al.* (2008). Prevalence of melanocortin-4 receptor deficiency in Europeans and their age-dependent penetrance in multigenerational pedigrees. Diabetes *57,* 2511-2518.

Suliman, A., Lam, A., Datta, R., and Srivastava, R.K. (2001). Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. Oncogene *20,* 2122-2133.

Sun, S., Ji, Y., Kersten, S., and Qi, L. (2012). Mechanisms of inflammatory responses in obese adipose tissue. Annu. Rev. Nutr. *32,* 261-286.

Tang, Q.Q., and Lane, M.D. (2012). Adipogenesis: from stem cell to adipocyte. Annu. Rev. Biochem. *81,* 715-736.

Tchkonia, T., Lenburg, M., Thomou, T., Giorgadze, N., Frampton, G., Pirtskhalava, T., Cartwright, A., Cartwright, M., Flanagan, J., Karagiannides, I., *et al.* (2007). Identification of depot-specific human fat cell progenitors through distinct expression profiles and developmental gene patterns. Am. J. Physiol. Endocrinol. Metab. *292,* E298-307.

Tchoukalova, Y., Koutsari, C., and Jensen, M. (2007). Committed subcutaneous preadipocytes are reduced in human obesity. Diabetologia *50,* 151-157.

Tchoukalova, Y.D., Votruba, S.B., Tchkonia, T., Giorgadze, N., Kirkland, J.L., and Jensen, M.D. (2010). Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. Proc. Natl. Acad. Sci. U. S. A. *107,* 18226-18231.

Torres-Leal, F.L., Fonseca-Alaniz, M.H., Rogero, M.M., and Tirapegui, J. (2010). The role of inflamed adipose tissue in the insulin resistance. Cell Biochem. Funct. *28,* 623-631.

Tran, K.V., Gealekman, O., Frontini, A., Zingaretti, M.C., Morroni, M., Giordano, A., Smorlesi, A., Perugini, J., De Matteis, R., Sbarbati, A., Corvera, S., and Cinti, S. (2012). The vascular endothelium of the adipose tissue gives rise to both white and brown fat cells. Cell. Metab. *15,* 222-229.

Trayhurn, P., Wang, B., and Wood, I.S. (2008). Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? Br. J. Nutr. *100,* 227-235.

Tsuchiya, S., Kobayashi, Y., Goto, Y., Okumura, H., Nakae, S., Konno, T., and Tada, K. (1982). Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. Cancer Res. *42,* 1530-1536.

Tsuchiya, S., Yamabe, M., Yamaguchi, Y., Kobayashi, Y., Konno, T., and Tada, K. (1980). Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int. J. Cancer *26,* 171-176.

Tung, Y.C., and Yeo, G.S. (2011). From GWAS to biology: lessons from FTO. Ann. N. Y. Acad. Sci. *1220,* 162-171.

Turer, A.T., and Scherer, P.E. (2012). Adiponectin: mechanistic insights and clinical implications. Diabetologia *55,* 2319-2326.

Van Gaal, L.F., Mertens, I.L., and De Block, C.E. (2006). Mechanisms linking obesity with cardiovascular disease. Nature *444,* 875-880.

Van Harmelen, V., Reynisdottir, S., Eriksson, P., Thorne, A., Hoffstedt, J., Lonnqvist, F., and Arner, P. (1998). Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes *47,* 913-917.

van Harmelen, V., Skurk, T., Rohrig, K., Lee, Y.M., Halbleib, M., Aprath-Husmann, I., and Hauner, H. (2003). Effect of BMI and age on adipose tissue cellularity and differentiation capacity in women. Int. J. Obes. Relat. Metab. Disord. *27,* 889-895.

van Loo, G., Saelens, X., van Gurp, M., MacFarlane, M., Martin, S.J., and Vandenabeele, P. (2002). The role of mitochondrial factors in apoptosis: a Russian roulette with more than one bullet. Cell Death Differ. *9,* 1031-1042.

van Loo, G., van Gurp, M., Depuydt, B., Srinivasula, S.M., Rodriguez, I., Alnemri, E.S., Gevaert, K., Vandekerckhove, J., Declercq, W., and Vandenabeele, P. (2002). The serine protease Omi/HtrA2 is released from mitochondria during apoptosis. Omi interacts with caspase-inhibitor XIAP and induces enhanced caspase activity. Cell Death Differ. *9,* 20-26.

van Marken Lichtenbelt, W.D., Vanhommerig, J.W., Smulders, N.M., Drossaerts, J.M., Kemerink, G.J., Bouvy, N.D., Schrauwen, P., and Teule, G.J. (2009). Cold-activated brown adipose tissue in healthy men. N. Engl. J. Med. *360,* 1500-1508.

Veilleux, A., Blouin, K., Rheaume, C., Daris, M., Marette, A., and Tchernof, A. (2009). Glucose transporter 4 and insulin receptor substrate-1 messenger RNA expression in omental and subcutaneous adipose tissue in women. Metabolism *58,* 624-631.

Virtanen, K.A., Lidell, M.E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N.J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. N. Engl. J. Med. *360,* 1518-1525.

Wajant, H., Pfizenmaier, K., and Scheurich, P. (2002). TNF-related apoptosis inducing ligand (TRAIL) and its receptors in tumor surveillance and cancer therapy. Apoptosis *7,* 449-459.

Wajchenberg, B.L. (2000). Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr. Rev. *21,* 697-738.

Walker, G.E., Marzullo, P., Verti, B., Guzzaloni, G., Maestrini, S., Zurleni, F., Liuzzi, A., and Di Blasio, A.M. (2008). Subcutaneous abdominal adipose tissue subcompartments: potential role in rosiglitazone effects. Obesity (Silver Spring) *16,* 1983-1991.

Walker, G.E., Verti, B., Marzullo, P., Savia, G., Mencarelli, M., Zurleni, F., Liuzzi, A., and Di Blasio, A.M. (2007). Deep subcutaneous adipose tissue: a distinct abdominal adipose depot. Obesity (Silver Spring) *15,* 1933-1943.

Wildman, R.P., Muntner, P., Reynolds, K., McGinn, A.P., Rajpathak, S., Wylie-Rosett, J., and Sowers, M.R. (2008). The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Arch. Intern. Med. *168,* 1617-1624.

Wronska, A., and Kmiec, Z. (2012). Structural and biochemical characteristics of various white adipose tissue depots. Acta Physiol. (Oxf) *205,* 194-208.

Wu, H., Ghosh, S., Perrard, X.D., Feng, L., Garcia, G.E., Perrard, J.L., Sweeney, J.F., Peterson, L.E., Chan, L., Smith, C.W., and Ballantyne, C.M. (2007). T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. Circulation *115,* 1029-1038.

Wynes, M.W., Frankel, S.K., and Riches, D.W. (2004). IL-4-induced macrophage-derived IGF-I protects myofibroblasts from apoptosis following growth factor withdrawal. J. Leukoc. Biol. *76,* 1019-1027.

Yang, Y.K., Chen, M., Clements, R.H., Abrams, G.A., Aprahamian, C.J., and Harmon, C.M. (2008). Human mesenteric adipose tissue plays unique role versus subcutaneous and omental fat in obesity related diabetes. Cell. Physiol. Biochem. *22,* 531-538.

Yarmo, M.N., Landry, A., Molgat, A.S., Gagnon, A., and Sorisky, A. (2009). Macrophage-conditioned medium inhibits differentiation-induced Rb phosphorylation in 3T3-L1 preadipocytes. Exp. Cell Res. *315,* 411-418.

Zha, B.S., and Zhou, H. (2012). ER Stress and Lipid Metabolism in Adipocytes. Biochem. Res. Int. *2012,* 312943.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J.M. (1994). Positional cloning of the mouse obese gene and its human homologue. Nature *372,* 425-432.

Zimmerlin, L., Donnenberg, V.S., Pfeifer, M.E., Meyer, E.M., Peault, B., Rubin, J.P., and Donnenberg, A.D. (2010). Stromal vascular progenitors in adult human adipose tissue. Cytometry A. *77,* 22-30.

Zuk, P.A., Zhu, M., Ashjian, P., De Ugarte, D.A., Huang, J.I., Mizuno, H., Alfonso, Z.C., Fraser, J.K., Benhaim, P., and Hedrick, M.H. (2002). Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell *13*, 4279-4295.

## 6.0 Curriculum Vitae