7-1-2013

Inflammatory cytokines alter normal lipid mobilization in adipocytes.

Nicholas Card

Nicholas Steven Card
Candidate

Biomedical Sciences
Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Dr. Robert Orlando, Chairperson

Dr. William Garver

Dr. Yijuan Sun

INFLAMMATORY CYTOKINES ALTER NORMAL LIPID MOBILIZATION IN ADIPOCYTES.

by

NICHOLAS STEVEN CARD

B.S. CHEMISTRY & BIOLOGY
UNIVERSITY OF NEW MEXICO, 2007

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science

Biomedical Sciences

The University of New Mexico
Albuquerque, New Mexico

July, 2013

DEDICATION

I would like to dedicate this to two people that have most driven my desire for lifelong learning and have developed my passion for science, and scientific reasoning. The first is Mrs. Leach, my high school genetics teacher. She showed me that I can be successful even with challenging course work and that pursuit and patience is more important than the grade itself. The other individual I would like to dedicate this to is my Sensei and Master Instructor, Zane Church. He taught me the value of hard work, knowledge, and perseverance.

ACKNOWLEDGMENTS

I would like to take a moment to acknowledge several people who have made this possible. I would first like to thank Dr. Robert Orlando, my mentor, for all the time, advice, input, guidance, support, and encouragement that he has provided. Even before I was in his lab he was going out of his way to give me guidance on a study abroad opportunity I was interested in. His passion for teaching, education, and the learning process has made an impression on me and increased my desire to also help educate and teach others. I would like to thank him for allowing me the chance to sit through the gastrointestinal (GI) medical block to gain a better understanding of clinical biochemistry.

In addition to my mentor, I would like to thank my committee, Dr. William Garver and Dr. Yijuan Sun, for their immense help and support. I would like to thank Dr. Garver for the opportunity to work in collaboration with him on the Niemann-Pick Disease Type C1 mouse project as well as work with his lab group, Dr. David Jelinek and Joseph Castillo.

I'm very thankful for an individual who has been there every step of the way and has gone out of her way to help me, teach me, and who has always been ready to listen and problem-solve with me, Dr. Carolina Franco Nitta. She has been a tremendous support, and I owe a lot to her for all of her help. She has been a great friend, tremendous resource, as well as teacher to me.

Lastly, I would like to thank my family and friends. It is with their support and encouragement that I was able to take on this challenge and succeed.

INFLAMMATORY CYTOKINES ALTER NORMAL LIPID MOBILIZATION IN ADIPOCYTES.

by

Nicholas Steven Card

B.S., Chemistry and Biology, University of New Mexico, 2007

ABSTRACT

Obesity is an increasing trend within the United States and the importance of addressing both causation and effects of obesity are becoming more important. It has been shown that environment, genetics, and social behavior factors can lead to an increased risk of obesity. Obesity has also been associated with several negative health concerns including increased risk for heart disease, cancer, poor nutrition, and diabetes, among others. Beyond identifying individual factors that may lead to obesity, and be associated with it, it is important to take into account complex obese biological systems which may have multiple factors compounding any health problems. Evidence has shown that obese adipose tissue can develop a state of chronic low grade inflammation with the presence of pro-inflammatory cytokines. Normal physiological agents, such as β-adrenergic agonists (for example epinephrine), can induce lipolytic function, though it has now also been shown that these pro-inflammatory cytokines can also stimulate lipolysis. To begin addressing the more complex issue of multiple obesity-related factors that contribute to health problems, we looked at a direct multi-factor based compounding system. This

system assessed the impact of multiple pro-inflammatory cytokines (Tumor Necrosis Factor-alpha and Interleukin-6) in combination with a β-adrenergic agonist (isoproterenol) to determine any combinatory effects on lipolytic function. Another biological system we used to assess lipolytic regulation was that of a disease based mouse model (Niemann-Pick Disease Type C1 Carrier – NPC1 +/-) which has been shown to affect cholesterol transport and cause weight gain and even obesity. We assessed the lipolytic impact that both of these systems may have. In the first system, assessments showed that pro-inflammatory cytokines, in combination with a β-adrenergic response, can induce an additive lipolytic stimulation. In the second system, decreased NPC1 gene dosage appeared to have an impact on Hormone Sensitive Lipase (HSL) mRNA levels and trends toward a decrease in glycerol release, an effective measure of lipolytic function. In all, these findings demonstrate that complex obese biological systems present multiple factors (either direct stimulating agents or indirect disease mechanisms) that can have substantial effects on lipolytic function.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INFLAMMATORY CYTOKINES ALTER NORMAL LIPID MOBILIZATION IN ADIPOCYTES

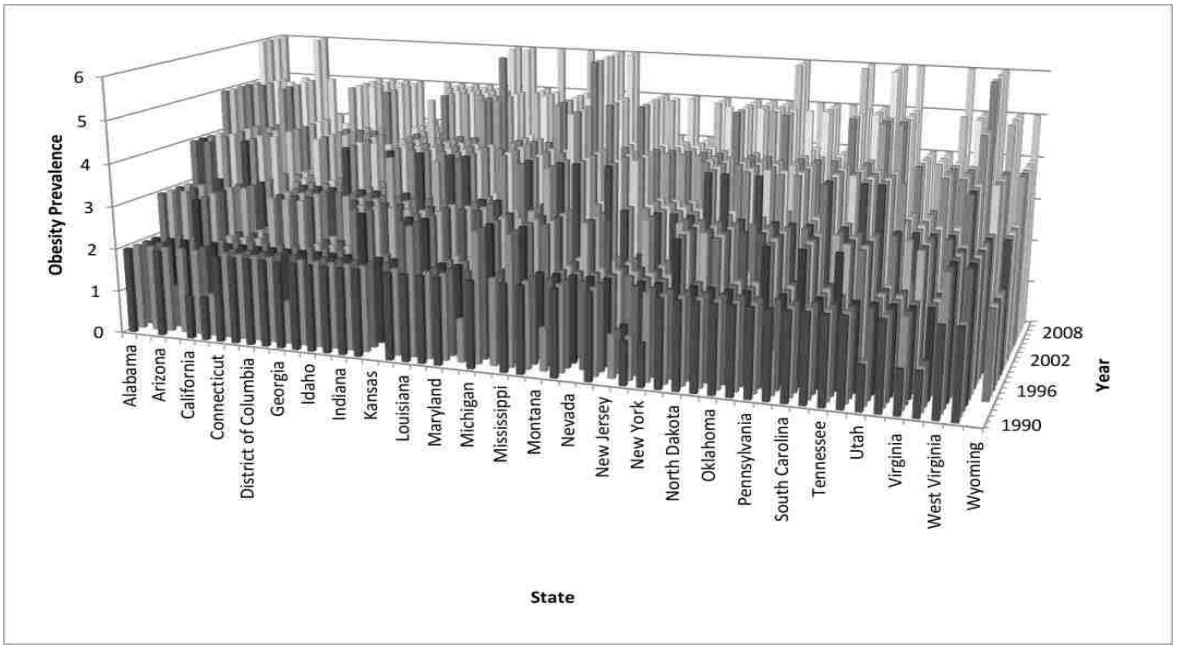
Chapter 1: Overall Introduction:

1.1 Body Fatness & Health

Since first finding methods to characterize body fatness, links between body fatness and a variety of health related issues has been discovered. The study of body fatness and health has grown in both breadth and depth. It encompasses a variety of academic disciplines such as: exercise science, nutrition, medical science, pharmacy, even reaching into policy and social and behavioral sciences. (Bray & Popkin, 1998; Clark, Goyder, Bissell, Blank, & Peters, 2007; Craig, Garthwaite, & Holloszy, 1987; Glueck et al., 2001; Hankey, Eley, Leslie, Hunter, & Lean, 2004; Jaime & Lock, 2009; Popkin & Gordon-Larsen, 2004) The depth of which can be seen through the amount of funding for some of these disciplines by the National Institute of Health (NIH). Over the past 4 years (2009 to 2012) NIH has allotted an average of $1.48 billion dollars towards nutrition research. Beyond that they have allotted an average of $1.05 billion dollars towards Diabetes research, and $809 million towards Obesity research, over that same time period. Over the next two years (2013 and 2014) the average amounts for each category will even exceed these numbers. ("NIH Categorical Spending -NIH Research Portfolio Online Reporting Tools (RePORT)," n.d.)

1.2 Body Mass Index

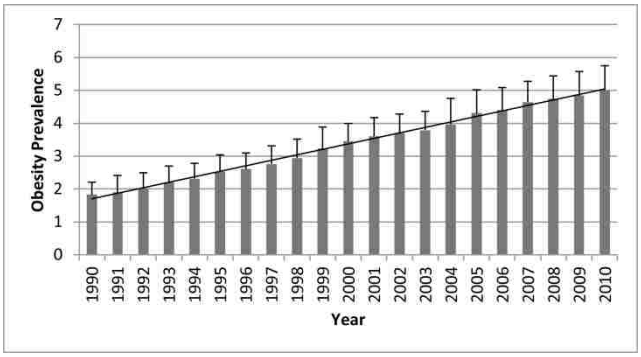
Body mass index (BMI) has become the reliable indicator used to determine a healthy body fatness range. ("Assessing Your Weight and Health Risk," n.d.-a, "Healthy Weight: Assessing Your Weight: Body Mass Index (BMI) | DNPAO | CDC," n.d.) This



method takes an individual's body mass and divides it by the square of their height. It was first developed as the result of understanding why there was a correlation between weight and cardiovascular disease. This information came after actuaries began reporting an increase in mortality among overweight insurance policyholders following World War II. (Eknoyan, 2008) Since this was first noticed, our understanding of the correlation has grown tremendously, yet we also recognize that we are far from fully understanding and implementing such knowledge to identify treatments for the obesity problem.

1.3 Obesity & Overweight Defined

Obesity and overweight are defined as ranges of weight that are greater than what is considered healthy at a given height. Overweight is defined as a BMI of 25 to 29.9 for a given height and weight for adults. Obesity is defined as a BMI of greater than or equal to 30 for a given height and weight for adults. ("Obesity and Overweight for Professionals: Adult: Defining - DNPAO - CDC," n.d.)



The Center for Disease Control & Prevention (CDC) has released information compiling obesity prevalence state by state every year. This information has been pivotal in recognizing we are far from fully understanding and implementing knowledge to combat the problem. After an assessment of the previous 20 years (1990 through 2010) of information gathered by the CDC, it can be seen that there is a dramatic increase in the prevalence of obesity for each individual state (Figure-1.1). As a whole, the US demonstrates a steady and rapid increase in obesity between 1990 and 2010 (Figure-1.2). ("Obesity and Overweight for Professionals: Data and Statistics: Adult Obesity - DNPAO - CDC," n.d.-a)

1.4 Causes of Obesity

Obesity is said to be caused by a complex interplay between the environment, genetics, and behavior. (Nguyen & El-Serag, 2010a) Environmental factors causing obesity are often explained in a simple manner as being the result of energy intake in excess of energy expenditure. These both independently have been shown to lead to obesity and certainly in combination have that ability. (William H. Dietz & Gortmaker, 1985; Kant & Graubard, 2006a; Andrew M. Prentice & Jebb, 1995) Energy intake commonly refers to consuming more food than required by the body to meet its daily needs. Daily energy need consists of three components: the resting metabolic rate, diet-induced thermogenesis, and activity-induced thermogenesis. (Donahoo, Levine, & Melanson, 2004a) The resting metabolic rate (RMR; also known as basal metabolic rate) is the cellular nutrition requirement necessary to maintain normal cellular function during rest. Diet-induced thermogenesis (also known as the thermic effect of food) is the nutrition requirement of the body for the processes involved in the break down, digestion, and absorption of food particles. Activity-induced thermogenesis is the nutrition requirements of the body in order to perform both exercise and non-exercise activities. Diet-induced thermogenesis typically accounts for approximately 5% to 15% of an individual's daily energy expenditure. (Westerterp, 2004) In combination, all three energy expenditure components should be equal with energy intake as a means to maintain weight balance.

Beyond environmental factors, genetic studies have demonstrated that there are strong correlations between genes and obesity. One gene of particular interest is the obesity-associated gene (FTO rs9939609). In a genome-wide association study of 38,579

patients, Frayling *et al.* found that the incidence of obesity is increased by 1.67-fold in patients that were homozygous for the FTO gene. (Andreasen et al., 2008a; Frayling et al., 2007a; Hunt et al., 2008a) As of the 12th update on the human obesity gene map, 253 quantitative trait loci (genetic-phenotype link) for human obesity related phenotypes had been identified. (Rankinen et al., 2006) One study also found an association with obesity and Niemann Pick Disease Type C1 (NPC1), a gene of interest which is discussed later in chapter 3. (Meyre, Delplanque, Chèvre, Lecoeur, Lobbens, Gallina, Durand, Vatin, Degraeve, Proença, et al., 2009a) Overall, a great deal of effort is being put into further researching possible genetic links with obesity and still more studies are needed to further illuminate the connection genetics has with obesity.

Lastly, behavior has also been shown to be linked with obesity. It has been suggested that those individuals with a friend who is obese have a 57% increased risk of becoming obese, those with a sibling who is obese had a 40% increased risk, and those with a spouse who is obese had a 37% increased risk. (Nguyen & El-Serag, 2010a) This indicates that one's social interactions may impact their risk perhaps in an inadvertent manner.

1.5 Fat Metabolism, Obesity, Health Correlations & Effects

Studies have shown that obesity and overweight are significantly correlated with several health concerns including: diabetes, high blood pressure, high cholesterol, as well as an overall poor health status.("Assessing Your Weight and Health Risk," n.d.-b; Mokdad AH, 2003) Associations between obesity, macrophage infiltration, and chronic

low-grade inflammation have also been made. (Galic, Oakhill, & Steinberg, 2010a; Kanda et al., 2006; Kershaw & Flier, 2004a; Xu et al., 2003a) Obese individuals demonstrate an increase of secreted pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNFα), Interleukin-6 (IL-6), Interferon-gamma (INF-γ), etc. (X.-H. Chen et al., 2010a; Feingold, Doerrler, Dinarello, Fiers, & Grunfeld, 1992a; Weisberg et al., 2003a) Additionally, cytokines can stimulate fatty acid release from adipose leading to an increase systemic fatty acid concentration (Hyperlipidemia). (X. Chen, Xun, Chen, & Wang, 2009a; Ji et al., 2011a; Nieto-Vazquez et al., 2008; Sandra C. Souza et al., 1998)

Hyperlipidemia is when abnormally elevated levels of lipids are present. If these levels increase enough, lipotixicity can occur. Lipotoxicity is the term used to describe a state in which adverse effects result from fatty acid accumulation in non-adipose tissues. Research has shown that lipid accumulation in tissues such as the heart, liver, kidneys, pancreas, as well as skeletal muscle can lead to the pathogenesis resulting in heart failure, obesity, and diabetes. These findings have been demonstrated in both mouse and human models. Additional lipotoxicity findings include cellular signaling dysfunction, and even apoptosis. (Perez-Martinez, Perez-Jimenez, & Lopez-Miranda, 2010a; Schaffer, 2003a; Unger, 2002a)

1.6 Obesity & Fat Metabolism

When looking at obesity, the metabolism of carbohydrates and lipids both have drawn a great deal of interest. Both carbohydrate and fat nutrients are a primary concern for weight gain and risk of obesity. Study of carbohydrate biochemistry has shed some

light on the role it plays in connection with obesity and BMI. Assessments have looked at associations between glycemic load, glycemic index, as well as quality of carbohydrate intake. Whole-grain intake has been shown to be inversely proportional to BMI, while refined grain intake appears not to be. (van Dam & Seidell, 2007) High glycemic load seems to be linked with lower BMI, while studies performed on glycemic index and its link with BMI appears to be less consistent. (Gaesser, 2007) Lipid biochemistry is also being looked at for links with obesity. Cross-sectional studies generally demonstrate that the concentration of fat in the diet positively correlates with relative body mass. Furthermore, intervention studies have shown that there is a consistent but short-lived weight loss experienced by individuals placed on a low-fat diet. (Lissner & Heitmann, 1995) Due to weight gain being closely correlated with insulin sensitivity, particularly in the abdominal region, recent interest has been brought to the quality of dietary fats. Of particular importance for insulin sensitivity seems to be a diet lower in saturated fatty acids and at least partially replaced, in appropriate situations, with mono- and polyunsaturated fatty acids. (Riccardi, Giacco, & Rivellese, 2004) From these and other studies, it is clear that the metabolism of carbohydrates and lipids can have a substantial impact on obesity and obesity-related health concerns.

Lipid biochemistry is a complex field of study that includes many unique and interconnected pathways. Lipid biochemistry can be divided into two general topic areas: anabolism (biosynthesis) or catabolism (degradation of macromolecules). This includes fatty acid synthesis and degradation, cholesterol biochemistry, triglyceride synthesis (fat storage) and degradation (energy mobilization), lipoprotein biochemistry (lipids coupled to proteins – commonly used for transport of cholesterol and triglycerides), and

membrane lipid biochemistry. (Styer, Tymoczko, & Berg, 2011) Obesity is largely defined as an excess storage of fat in the form of triglycerides, making characterization of those processes centering on the synthesis and degradation of triglycerides of central importance to developing a better understanding of obesity. ("Obesity: MedlinePlus," n.d.)

1.7 Fat Metabolism Pathways

The process of fat storage by adipose tissue requires precursors of fatty acids and glycerol, lipogenesis (combining fatty acids and glycerol to synthesize triglycerides), and is balanced by the reverse process of lipolysis (the degradation of stored triglycerides). Transport and translocation of fatty acids is key to the fat storage pathways allowing for proper availability of triglyceride constituents. Albumin is a key systemic transport protein allowing for fatty acid delivery to storage tissues such as the white adipose tissue by overcoming solubility difficulties in plasma being a highly hydrophilic environment. (Abumrad, Harmon, & Ibrahimi, 1998) Short chain fatty acids can diffuse through lipid membranes, though long chain fatty acids require support proteins for this process. A series of proteins known as Fatty Acid Binding Proteins (FABP) and Fatty Acid Transport Proteins (FATP) along with CD36 are the primary proteins in this process. (Large, Peroni, Letexier, Ray, & Beylot, 2004a; Pelsers, Stellingzoerff, & Van Loon, 2008a) The liver also makes triglycerides through the lipogenic processes and transports these triglycerides to other tissues for use through via lipoproteins, chylomicrons, as well as very low-density lipoproteins (VLDL). When VLDL is transported to adipose tissue,

Lipoprotein Lipase (LPL) activity releases fatty acids from VLDL particles to permit transport into the cell. (Preiss-Landl, Zimmermann, Hämmerle, & Zechner, 2002; Wang & Eckel, 2009)

Lipogenesis is the pathway involved with de novo synthesis of fatty acids from acetyl-CoA precursors. This pathway is activated in response to excess dietary carbohydrate intake, particularly that of glucose. Both the liver as well as adipose tissue have been shown to perform lipogenesis with liver being the greatest contributor. (Hems, Rath, & Verrinder, 1975; Large, Peroni, Letexier, Ray, & Beylot, 2004b) A key enzyme complex for the synthesis of fatty acids is fatty acid synthase (FAS). (Large et al., 2004b) Fatty acids are synthesized through a series of repetitive reactions, including condensation, reduction, and dehydration reactions. (Styer et al., 2011) The result of these series of reactions is a 16 carbon fatty acid, termed palmitate. Through the process of lipogenesis, de novo fatty acid synthesis can provide a source of fatty acids for immediate use by tissues for energy production or for long-term storage for energy needs at a later time.

Triglyceride synthesis is the production of the storage form of fat. The predominant storage site for triglycerides is in white adipose tissue. ("Adipose tissue. In: Encyclopedia of Sports Medicine and Science - Google Scholar," n.d.; Large, Peroni, Letexier, Ray, & Beylot, 2004c; Zechner, Kienesberger, Haemmerle, Zimmermann, & Lass, 2009a) Fatty acid availability may be the result of lipogenic processes or from oral intake of short-chain or long-chain fatty acids. Synthesis of triglycerides is performed through a series of acylation reactions using fatty acyl-CoA coupling these toa glycerol 3-phosphate backbone Glycerol 3-phosphate is actively synthesized within adipocytes in

glycolysis. Additionally, three fatty acyl-CoAs are required to construct a triglyceride. (Styer et al., 2011) Following the triglyceride synthesis, support proteins participate in storing triglycerides in the form of a cytosolic lipid droplet within the adipocyte until fatty acids are needed during times of whole body energy depletion. (Murphy, Martin, & Parton, 2009; Olofsson et al., 2008)

The lipolytic pathway mobilizes fatty acids from storage depots in adipose tissue, in order to provide systemic availability of fatty acids and glycerol to accommodate energy needs. A series of enzymes and regulatory proteins provide this function. Recent finding have demonstrated that Adipose Triglyceride Lipase (ATGL) is the predominant enzyme involved in the first step of fatty acid release from the triglyceride (three attached fatty acids) to the diglyceride (two attached fatty acids) form. Regulatory proteins have been identified that are critical to activation and control of lipolytic activity, in particular the lipid droplet coat protein Comparative Gene Identification-58 (CGI-58). CGI-58 is necessary for activation of ATGL. Afterwhich, Hormone Sensitive Lipase (HSL) then continues the second step of the degradation process, being the primary enzyme involved in diglyceride to monoglyceride (one fatty acid attached to the glycerol backbone) degradation. The final step of triglyceride degradation involves the enzyme Monoglyceride Lipase (MGL), which cleaves the last fatty acid from the glycerol backbone, hydrolyzing the monoglyceride into its constituents. (Lass, Zimmermann, Oberer, & Zechner, 2011a; Zechner, Kienesberger, Haemmerle, Zimmermann, & Lass, 2009b) Another key regulatory protein that is involved in lipolytic function is another lipid droplet coat protein known as perilipin. Recent findings have demonstrated that multiple perilipin isoforms are present on lipid droplets. The exact function of these

different isoforms is still being determined. Evidence has shown that perilipins can restrict access to the lipid droplet surface preventing lipolytic function of cytosolic lipases, such as ATGL and HSL. (Subramanian et al., 2004a; Wang et al., 2011) In all, the lipolytic pathway for mobilization of fatty acids from the storage form is a complex interplay between lipases and regulatory proteins, of which many of their interactions are still incompletely understood.

1.8 Regulation of Fat Metabolism

Regulation of fat metabolism is an important component to health. Several common regulators are known for their impact on fat metabolism. In a fed state, insulin functions to stimulate the uptake of nutrients from the circulation, such as carbohydrates, fats, and proteins, in order to store them for later use. (Foufelle & Ferré, 2002; Jaworski, Sarkadi-Nagy, Duncan, Ahmadian, & Sul, 2007a; Koo, Dutcher, & Towle, 2001; Large, Peroni, Letexier, Ray, & Beylot, 2004d) Besides facilitating nutrient uptake, insulin is able to stimulate both glycogen and fat synthesis, as well as act as a potent inhibitor of lipolysis. (Degerman et al., 1998)

Glucagon is the hormonal signal for energy mobilization (during a fasting state) and has been shown to stimulate lipolysis. (Bertrand, Masoro, & YU, 1980; Brubaker & Drucker, 2002; HECKEMEYER, BARKER, DUCKWORTH, & SOLOMON, 1983) A more potent stimulator of lipolytic function than glucagon is β-adrenergic signaling, seen during an exercise state.

Catecholamines, in particular epinephrine and norepinephrine, are the major hormonal regulators of the lipolytic processes. There are three receptors named β1, β2, and β3-adrenergic receptor, which respond to catecholamines inducing increased lipolytic response. (Jaworski, Sarkadi-Nagy, Duncan, Ahmadian, & Sul, 2007b; Large, Peroni, Letexier, Ray, & Beylot, 2004e) Upon catecholamine activation of the β-adrenergic receptors, lipid droplet coat protein perilipin becomes phosphorylated by a cAMP-dependent protein kinase (PKA), thus facilitating docking of lipases. (Subramanian et al., 2004b) Lipolytic response is species and receptor specific for β-adrenergic receptors, with β3-adrenergic receptors inducing the largest lipolytic response in rodents. (Langin, 2006a; Zechner, Kienesberger, Haemmerle, Zimmermann, & Lass, 2009c) Isoproterenol is an effective artificial β3-adrenergic receptor agonist and stimulates lipolysis in rodents. (Anthonsen, Rönnstrand, Wernstedt, Degerman, & Holm, 1998; Zimmermann et al., 2004) For my studies, isoproterenol is used as a β3-adrenergic receptor agonist to mimic a physiological catecholamine response.

Other agents have also been shown to be effective at stimulating or inhibiting fat metabolism. Additional lipolytic stimulants include thyroid hormone, growth hormone, as well as natriuretic peptide. Obesity-dependent pro-inflammatory cytokines secreted into the local environment of adipose tissue have also been shown to stimulate lipolytic function including TNFα and IL-6. (Chaves, Frasson, & Kawashita, 2011a) Agents that are able to inhibit fat mobilization in some fashion include neuropeptide Y as well as adenosine. (Jaworski, Sarkadi-Nagy, Duncan, Ahmadian, & Sul, 2007c) The importance of understanding normal non-obese lipolytic regulation allows for improved recognition of the serious nature of compounding lipolytic effects seen in an obese system.

1.9 Detrimental Lipolytic Regulation

The possibility for lipolytic dysregulation is becoming more important for the field of obesity research. Up until recently, white adipose tissue (WAT) was thought to be primarily just a storage site for fat. As health concerns such as obesity, type II diabetes, and coronary heart disease have increased, further study of WAT has brought to light new information increasing the implications that poor regulation of WAT activity may have on an individual's health. An important finding was that obese WAT exhibits an inflammatory component. This inflammatory response includes adipose tissue's ability to express pro-inflammatory cytokines such as TNFα, IL-6, among others. (Galic et al., 2010a; Kershaw & Flier, 2004a) It has also been shown in genetically obese mouse models or mice made obese by a high fat diet that a large number of macrophages are present in WAT. (Xu et al., 2003a) With the discovery of pro-inflammatory cytokines in WAT, it has also been revealed that they in turn have an impact on fat metabolism. (X.-H. Chen et al., 2010a; Jaworski, Sarkadi-Nagy, Duncan, Ahmadian, & Sul, 2007d; Ji et al., 2011b; Langin & Arner, 2006a; Zechner, Kienesberger, Haemmerle, Zimmermann, & Lass, 2009d)

Chapter 2: Effects of combined inflammatory cytokine and β-adrenergic stimulation on lipid mobilization in 3T3-L1 adipocytes:

2.1 Introduction

2.1.1 Lipolysis & Normal Physiology

Lipolysis is an important pathway for individuals allowing for proper fat mobilization and homeostasis. Several agents have been shown to have the ability to stimulate lipolysis including: catecholamines, natriuretic peptide, and even glucagon. (Chaves, Frasson, & Kawashita, 2011b) These agents allow for proper mobilization during depleted energy states as well as periods of anabolic need. The roles of agents such as adrenergic agonists, for example epinephrine and isoproterenol, as well as agents such as glucagon have been pursued to better recognize their effect on lipolysis. Glucagon is the hormone most commonly associated with stabilizing low blood glucose levels. Glucagon's effectiveness as a lipolytic stimulator has also been shown. Catecholamines are potent stimulants of the lipolytic pathway and are frequently used to study the pathway. (Jaworski, Sarkadi-Nagy, Duncan, Ahmadian, & Sul, 2007e) Isoproterenol is used in these studies to mimic a lipolytic response to a physiological β-adrenergic agonist.

2.1.2 Pathophysiology & Inflammation

Studies have shown that adipose tissue demonstrates properties of an endocrine-like gland. (Galic, Oakhill, & Steinberg, 2010b; Kanda et al., 2006; Weisberg et al., 2003b) As part of this finding, it has been established that adipose tissue of obese individuals expresses an increased amount of pro-inflammatory proteins that can cause a chronic low grade inflammation. (Fried, Bunkin, & Greenberg, 1998; Kanda et al., 2006; Perreault & Marette, 2001; Samad, Yamamoto, Pandey, & Loskutoff, 1997; Sartipy & Loskutoff, 2003; Visser M, 1999; Weyer et al., 2002) Independent of other problems related to an increased adipose volume, pro-inflammatory cytokines alone have been linked with a variety of negative health issues such as: hyperlipidemia, insulin resistance, and even with poor sleep regulation resulting in fatigue. (Feingold, Doerrler, Dinarello, Fiers, & Grunfeld, 1992b; Vgontzas et al., 1997) One mechanism which is being carefully considered in relation to several of these health issues is that of the impact of pro-inflammatory cytokines upon the lipolytic pathway. Of particular importance is the ability of pro-inflammatory cytokines to be potent stimulators of fatty acid mobilization. (Feingold et al., 1992b)

2.1.3 TNFα & IL-6

Two increasingly significant pro-inflammatory cytokines, Tumor Necrosis Factor alpha (TNFα) and Interleukin-6 (IL-6), have been associated with increased lipolytic stimulation. Studies have found that both TNFα and IL-6 are increased with obese non-diabetics and diabetics as compared to lean controls. (Bastard et al., 2000)

Research on TNFα's impact upon adipose metabolism has been found to be multi-fold in facet. Clinically, TNFα has been linked with obesity, insulin resistance, as well as with chronic inflammation. (Hotamisligil, Shargill, & Spiegelman, 1993; Nieto-Vazquez et al., 2008) Mechanistically, TNFα influences a variety of components of adipose metabolism including: uptake of fatty acids, the lipogenic and lipolytic pathways, and even enzyme activity and adipokine regulation. (X. Chen et al., 2009a) Lipolytic stimulation by TNFα has revealed it to be a potent agent in dysregulating lipid homeostasis in an inflamed state. (Sandra C. Souza et al., 1998)

Recent research has begun to shed light on how involved IL-6 is in adipose metabolism; however, unlike TNFα, IL-6's impact on adipose metabolism is not as well understood. Initial research has indicated IL-6 is connected to obesity and obesity related health concerns. An inverse correlation has been shown between adipose tissue IL-6 content and insulin-responsiveness. (Bastard et al., 2002) Additionally, weight loss has been shown to decrease IL-6 levels in human subjects. (Bastard et al., 2000) Evidence has been presented suggesting that IL-6 is capable of stimulating lipolytic function. (Ji et al., 2011a) Cytokines appear to be a key factor in mechanistic action that can correlate obesity with many of its associated health concerns.

2.1.4 Hypothesis & Specific Aims

Complex biological systems have multiple lipolytic stimulating agents available in an obese individual including inflammatory cytokines and beta-adrenergic agonists. (Galic, Oakhill, & Steinberg, 2010c; Weisberg et al., 2003a) Any significant increase in lipolytic stimulation may exacerbate a disease state, such as lipotoxicity. The ***hypothesis*** developed to evaluate this concept was that pro-inflammatory cytokines, such as TNFα and IL-6, augment β-adrenergic stimulation of the lipolytic pathway. To better determine the lipolytic stimulating power of adipocytes having access to multiple agents simultaneously, a 3T3-L1 cell culture model was used to assess cytokine and β-adrenergic co-stimulation, as well as a triple-stimulation state with multiple cytokines and β-adrenergic stimulation. 3T3-L1 adipocytes are an immortalized cell line that are commonly used in adipocyte research. Following a differentiation protocol (described in 2.2.1) to generate mature adipocytes, these cells store triglycerides in lipid droplets that are indistinguishable from normal adipocytes. In order to test this hypothesis, three specific aims were developed to investigate its validity.

Specific aim #1: To determine the individual quantitative effects of TNFα, IL-6 or isoproterenol on fatty acid mobilization in 3T3-L1-derived adipocytes.

Specific aim #2: To measure the quantitative effects of combining isoproterenol with TNFα OR IL-6 (dual stimulation) on fatty acid mobilization in 3T3-L1-derived adipocytes

Specific aim #3: To determine the quantitative effects of combining isoproterenol with TNFα AND IL-6 (triple stimulation) on fatty acid mobilization in 3T3-L1-derived adipocytes.

2.2 Methods

2.2.1 Cell Culture and Treatment

3T3-L1 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), grown and differentiated in complete Dulbecco's Modified Eagle Medium (DMEM) with high-glucose and supplemented with 10% fetal bovine serum (FBS). Cells were incubated at 37°C in a 5% CO_2 environment. Briefly, 12-well culture plates were coated with 1% gelatin prior to cell seeding. Cells were allowed to come to confluency, and then differentiated by incubating cells for 3 days with 450µM 3-isobutyl-1-methylxanthine, 250nM dexamethasone, and 167nM insulin, followed by 4 days supplementation with insulin alone. To confirm differentiation, visual inspection of cells was performed to assess for lipid droplet formation as well as morphology changes within the adipocytes. Experimentation occurred typically 7 days after induction of differentiation. Before 24 hour treatment periods, cells were incubated with media with only 10% FBS for a period of 24 hours unless otherwise specified. TNFα (Cell Signaling, Danvers, MA), isoproterenol (Sigma-Aldrich, Saint Louis, MO), or IL-6 (eBioscience, San Diego, CA) treatment was performed using either a time course treatment or a 24 hour treatment, as indicated.

2.2.2 Glycerol Release Measurement

Lipolytic determinations were made by assessing glycerol release in culture medium. (Aboulaich, Chui, Asara, Flier, & Maratos-Flier, 2011; Lamb, Goldsmith, Bennett, Finch, & Bell-Pedersen, 2011; Schleich & Teleman, 2009) Culture media was

collected and measured by spectrophotometer (Molecular Devices, SpectraMax Plus) at 540nm using free glycerol reagent (Sigma-Aldrich). Samples were compared to a glycerol standard solution (Sigma-Aldrich) to quantify glycerol release.

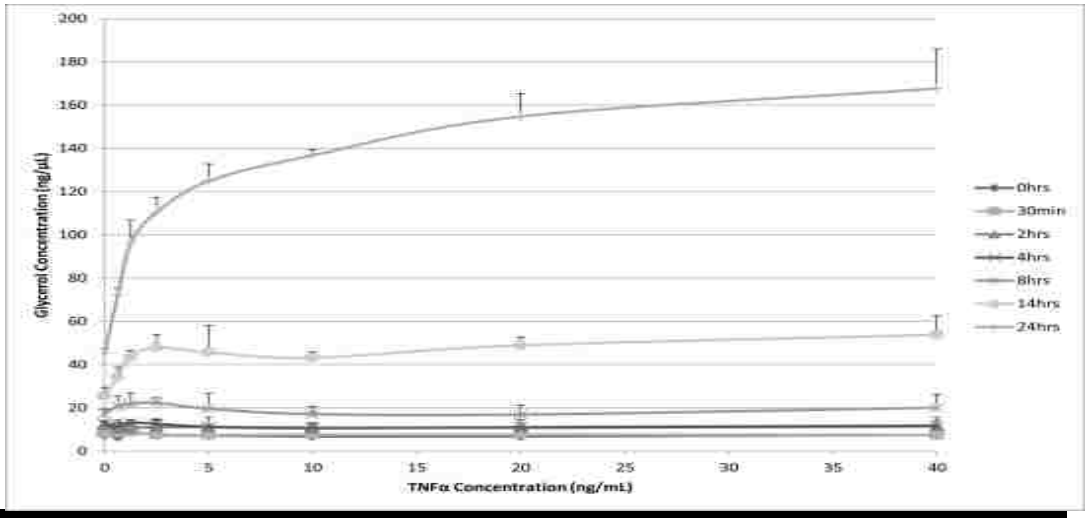
2.2.3 Statistical Analysis

Statistical significance was determined by performed t-tests of data in Microsoft Excel 2010. Statistical significance was determined if p-value < 0.05.

2.3 Results

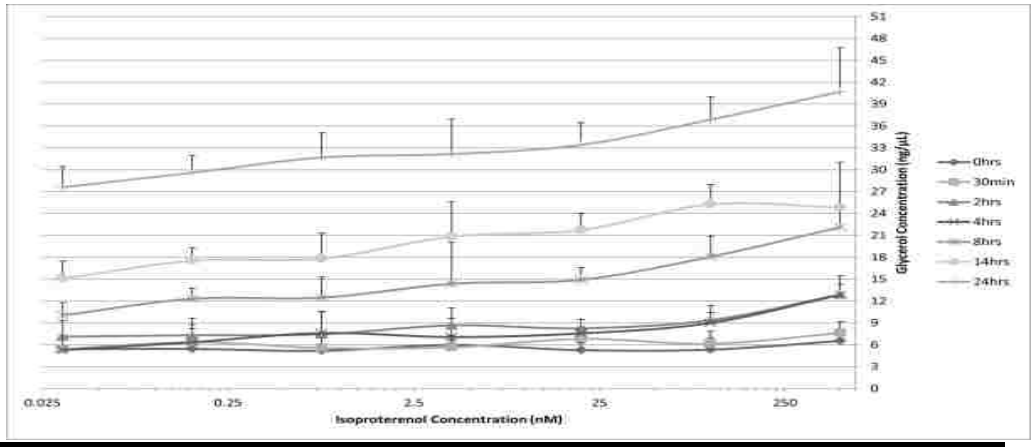
2.3.1 Lipid Mobilization

Fat mobilization has been shown to be mediated by several agents. Isoproterenol is a common β-adrenergic lipolytic stimulator. (Anthonsen et al., 1998; Zimmermann et



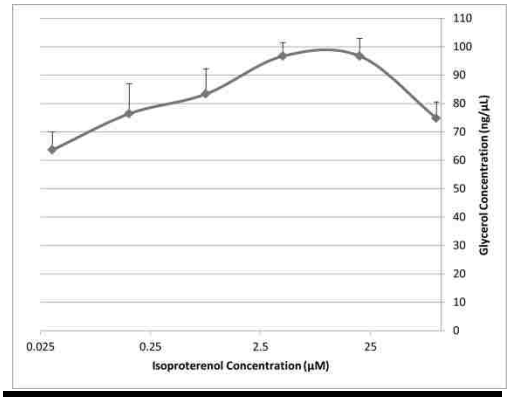
al., 2004) Recently, additional factors have been found to stimulate fat release, including: TNFα, IL-6, natriuretic peptide. (Chaves et al., 2011a) The process of fat release from a storage state can be observed using a glycerol release assay, as this indicates all three fatty acids have been released from the glycerol backbone.

To best deduce the overall impact on glycerol release, and to allow for signaling and transcriptional level events to occur, a time course was performed. Time courses were performed with varying concentrations for isoproterenol as well as TNFα, an optimal treatment course of 24 hours was determined (Figure-2.1, Figure-2.2). Additionally, 24 hours of treatment with IL-6 (Figure-2.4) yielded a relatively smaller degree of glycerol release, further substantiating the importance of a 24 hour treatment period.



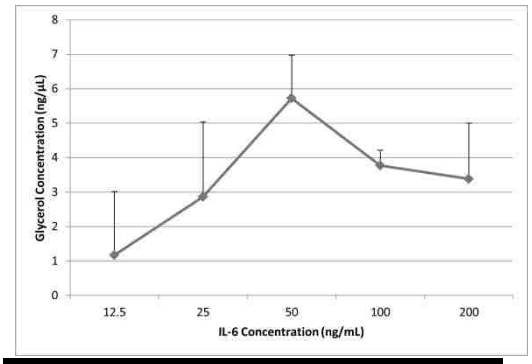
[redacted]

To best determine optimal stimulation, saturation curves were performed for each agent. Stimulation of 1µM isoproterenol demonstrated optimal glycerol release (Figure-2.3). Moreover, a concentration of 10ng/mL of TNFα (Figure-2.1) was shown to be optimal and 50ng/mL of IL-6 (Figure-2.4). Each agent demonstrated significance in comparison to stimulation above basal level (data not shown).



[redacted]

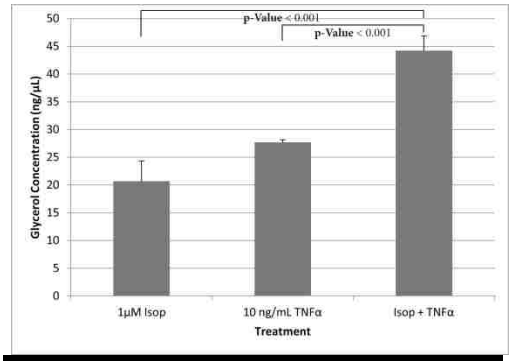
In addition, insulin has been shown to be a potent inhibitor of fat mobilization. (Degerman et al., 1998) To determine the post treatment inhibitory impact of insulin upon glycerol release, an insulin free media time course administration was performed (data not shown). This experiment was performed by maintaining a constant 24 hour treatment course, but having a 0, 24, 48, and 72 hour insulin free period where cells were rinsed free of insulin and maintained in medium without insulin. This data showed an optimal insulin free media course of 24 hours, followed by 24 hours of treatment with stimulating agents.



[redacted]

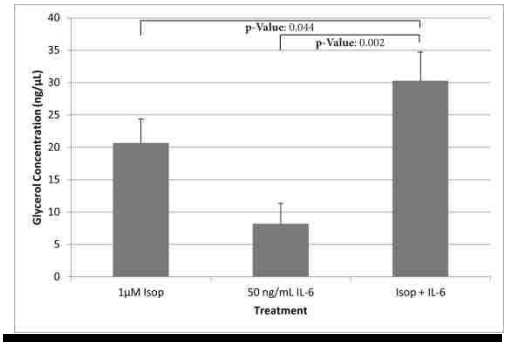
2.3.2 Co-Stimulation of Fat Mobilization

Inflammation has recently been attributed as a characteristic of obese adipose tissue. (Galic, Oakhill, & Steinberg, 2010d; Weisberg et al., 2003c) Inflammatory cytokines such as TNFα and more recently IL-6 have been shown to stimulate fat release in adipocytes. (X. Chen, Xun, Chen, & Wang, 2009b; Ji et al., 2011c; S C Souza et al., 1998) In complex biological systems, inflammatory



[redacted]

stimulating factors are not alone in their ability to mobilize fat. Normal physiological factors such as epinephrine (a β-adrenergic agonist) also have this ability. (Langin, 2006b; Large, Peroni, Letexier, Ray, & Beylot, 2004f; Subramanian et al., 2004c; Zechner, Kienesberger, Haemmerle, Zimmermann, & Lass, 2009e) To better understand these complex biological systems it is important to account for multiple factors being present simultaneously. To determine if stimulation of fat release is additive in nature, known inflammatory based stimulatory agents (TNFα and IL-6) were each independently compared to their respective co-stimulation with isoproterenol, a well-known β-adrenergic agonist.

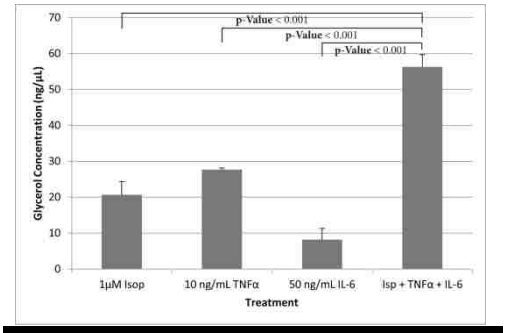


First, TNFα was assessed to determine if its supplemental impact on fat mobilization with that of isoproterenol was directly additive, partially augmented, or sustained. After 24 hours of stimulation, it was found that glycerol release demonstrated an additive effect (Figure-2.5). After subtracting basal level stimulation, independent glycerol concentration levels of isoproterenol were measured at 20.68ng/µL and TNFα at 27.7ng/µL. Taking the independent glycerol levels to be additive would yield a glycerol concentration of 48.38ng/µL. The TNFα and isoproterenol co-stimulation based glycerol concentration level was measured at 44.22ng/µL. The co-stimulation not only demonstrated statistical significance from independent stimulation, but also seemed to demonstrate a directly additive nature.

After TNFα, IL-6 was then assessed for its supplemental ability to stimulate fat release with isoproterenol. Again, after a 24 hour period of stimulation, glycerol release was measured and an additive effect was measured (Figure-2.6). Independent glycerol concentration levels were measured at 8.19ng/µL for IL-6 and isoproterenol was measured at 20.68ng/µL. These independent values taken to be additive would yield a value of 28.87ng/µL. The measured co-stimulation glycerol concentration was measured at 30.3ng/µL. As was seen with TNFα, co-stimulation of IL-6 and isoproterenol again showed statistical significance and seemed to demonstrate a directly additive nature.

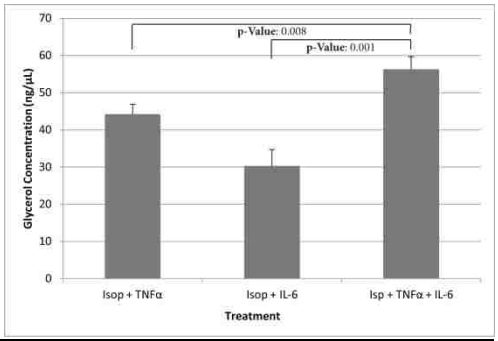
2.3.3 Triple-Stimulation of Fat Mobilization

To better deduce how much of an impact multiple factors can have on fat mobilization, an experiment was performed in which all three stimulating agents (isoproterenol, TNFα, and IL-6) were combined. This type of a system was again designed to be more demonstrative of a complex biological system, with multiple stimulating agents accessible to cells.



To first assess this triple-stimulation system, it was measured against each factor independently. Once basal level stimulation was removed, each of the three factors were measured for glycerol release. Measurements were 20.68ng/µL for isoproterenol, 27.7ng/µL for TNFα, and 8.19ng/µL for IL-6. Each factor taken independently and measured in an

additive manner would yield a glycerol concentration of 56.58ng/µL. The triple-stimulation (Figure-2.7) glycerol concentration was measured at 56.32ng/µL. This shows that the glycerol release by these three stimulatory agents yield results that appear to be directly additive in nature.



Additionally, two co-stimulation experiments, isoproterenol with TNFα and isoproterenol with IL-6, were compared to that of the triple-stimulation, isoproterenol with TNFα and IL-6. Through examination, a statistical increase was seen in the triple-stimulation from that of either co-stimulation (Figure-2.8). This supports the hypothesis that multiple stimulating agents have a compounding effect on glycerol release.

2.4 Discussion

Previous findings have demonstrated that obese adipose tissue maintains a chronic, low grade inflammation that is accompanied by the presence of pro-inflammatory cytokines such as TNFα and IL-6. (Galic, Oakhill, & Steinberg, 2010e) Normal physiological regulation of lipolytic processes are performed by factors such as catecholamines (adrenergic agonists), among others. (Jaworski, Sarkadi-Nagy, Duncan, Ahmadian, & Sul, 2007f) When a normal physiological regulation is supplemented by inflammatory regulatory factors, the end result appears to be significantly more potent activation of the lipolytic system than any single factor would independently.

When assessing true obese biological systems, all of these factors may be present in an individual, and therefore determining the impact of a compounded lipolytic stimulation may present even further detrimental effects on the system. Therapeutic agents acting to combat insulin resistance, hyperlipidemia, and other health concerns, must be able to take into account these compounding factors. Assessment of the hypothesis that pro-inflammatory cytokines, such as TNFα and IL-6, additionally and positively augment β-adrenergic stimulation of the lipolytic pathway showed that this appears to be the case in a 3T3-L1 model system.

Signaling level events may further elucidate the mechanism of action for this compounding lipolytic stimulation. In addition, it will be helpful to further evaluate the impact of a co- and triple-stimulation using a diet induced obesity rodent model to further elucidate the effects on the system after such stimulation.

Chapter 3: Glycerol Release in Niemann-Pick Disease Type C1 Carrier Mice:

3.1 Introduction

3.1.1 Niemann-Pick Disease

Niemann-Pick disease (NP) was first named from work performed by Albert Niemann and Ludwig Pick. It is classified as Sphingomyelinase deficiency. NP is a group of diseases that demonstrate lipid collection in organs such as the spleen, liver, brain, and even white adipose tissue. There are four subtypes of NP classified as Type A, B, C, and D. Each of these show a variety of symptoms based upon the organs impacted, whether there is nervous system involvement, and age of occurrence. ("Niemann-Pick disease - National Library of Medicine - PubMed Health," n.d.-a; Patterson et al., 2012a; Vanier, 2010)

3.1.2 Niemann-Pick Disease Type C

NP Type C (referred to as NPC) results when cholesterol and other lipids cannot be properly broken down and therefore leads to increased cholesterol in the liver and spleen and other lipid forms in the brain. NPC has two genes that have been associated with it, of which gene 1 (NPC1) is present in approximately 95% of cases, while gene 2 (NPC2) is present in only 5% of the cases. ("Niemann-Pick disease - National Library of Medicine - PubMed Health," n.d.-b; Patterson et al., 2012b) Symptoms for NPC1 typically include: enlarged liver, spleen, jaundice, learning difficulties, intellectual

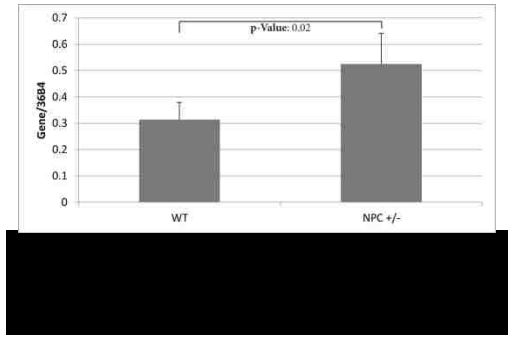
decline, seizures, slurred or irregular speech, tremors, difficulty moving limbs, and more. ("Niemann-Pick disease - National Library of Medicine - PubMed Health," n.d.-b)

3.1.3 Niemann-Pick Disease Type C1

The NPC1 gene codes for a membrane-bound protein that has multiple cholesterol binding regions. The NPC1 protein is associated with the late endosomes, lysosome, and *trans*-Golgi network and is believed to be involved in the transport of low density lipoproteins (LDL) derived cholesterol from the late endosome/lysosome to other cellular compartments. (Garver, Heidenreich, Erickson, Thomas, & Wilson, 2000; Higgins, Davies, Chen, & Ioannou, 1999; Jelinek et al., 2012a; Liscum, Ruggiero, & Faust, 1989; Meyre, Delplanque, Chèvre, Lecoeur, Lobbens, Gallina, Durand, Vatin, Degraeve, Proença, et al., 2009b)

3.1.4 NPC1 Carrier Mouse Model

In connection with NPC1's cholesterol and lipid link; the NPC1 gene has also now been associated with both human obesity and with weight gain in mice that have a decreased gene dosage. (Jelinek et al., 2012a; Jelinek, Heidenreich, Erickson, & Garver, 2010a) Beyond weight impact, the adipose tissue was also assessed for F4/80, a



macrophage specific antigen. (Wellen & Hotamisligil, 2003) As shown in Figure-3.1, NPC1 carrier (NPC1 +/-) status demonstrated a statistically significant increase in F4/80 as compared to wild-type. This finding shows that microphage infiltration is greater in adipose from NPC1 carrier mice than wild-type, which infers an increased likelihood that pro-inflammatory cytokines are present in the tissue. (Xu et al., 2003b) At this time the consequence of inflammatory-induced lipolytic stimulation in the context of NPC1 gene variants are still unknown.

3.1.5 Hypothesis & Specific Aims

To better understand the correlation between the obesity and NPC1, and possible effects on lipolytic function, we ***hypothesize*** that decreased NPC1 gene dosage (haploinsufficiency) results in increased fat pad weight by mechanisms that decrease lipolytic genes. To best test this hypothesis we pursued three specific aims.

Specific aim #1: To confirm the body weight differential previously published and to determine if differences in fat pad weight were also present.

Specific aim #2: To quantify lipolytic activity in wild type mice and NPC1 +/- mice by measuring glycerol release.

S***pecific aim #3***: To identify which genes involved in fat metabolism may be alternatively regulated by the NPC1 carrier status.

3.2 Methods

3.2.1 Mice

Male $NPC1^{+/+}$ and $NPC^{+/-}$ (BALB/cJ genetic background) mice were raised at the University of New Mexico Animal Research Facility following conditions reported previously. (Jelinek, Heidenreich, Erickson, & Garver, 2010b) Mice were raised with 4 mice per cage, at a temperature maintained between 73°- 75°F, humidity of 46%-52%, and light periods of 12-hour alternating light/dark cycle. Mice had free access to food and water. All mice were placed on a 16 week high-fat diet and weighed weekly until 160 days, when they were harvested. After the 160 day period, $NPC1^{+/+}$ mice weighed an average 35.2g and $NPC1^{+/-}$ mice weighed an average of 41.05g.

3.2.2 Fat Pad Excision

Following a 3-5 minute CO_2 euthanization process, fur of the abdominal section was cleaned with 70% alcohol solution and an incision approximately 2-cm in length was made. From this incision the fat pad was excised and testes were removed. Fat pads were weighed and placed in DMEM/high-glucose medium supplemented with 10% fetal bovine serum (FBS). Part of the fat pad was placed in 6mL RNALater and stored at -20°C for Quantitative Real-Time PCR analysis. The fat pad tissue in media was incubated at 37°C in a 5% CO_2 environment for a period of 24 hours.

3.2.3 Weight Normalization

Following a 24 hour incubation period, fat pad tissue was cut into whole pieces of approximate weight of 0.1g to 0.15g. Tissue was weighed and recorded to normalize glycerol release to the amount of tissue in each well.

3.2.4 Glycerol Release Assay

Lipolytic determinations were made by assessing glycerol release in culture medium following a 4 hour incubation period. (Aboulaich et al., 2011; Lamb et al., 2011; Schleich & Teleman, 2009) Culture media was collected and measured by spectrophotometer (Molecular Devices, SpectraMax Plus) at 540nm using free glycerol reagent (Sigma-Aldrich) as reported in chapter 2.2.2. Samples were compared to a glycerol standard solution (Sigma-Aldrich) to quantify glycerol release.

3.2.5 Quantitative Real-Time PCR

Mouse adipose tissue was homogenized using a tissue homogenizer. RNA was isolated using an RNAeasy Mini Kit (Qiagen, Valencia, CA) following the manufacturers recommendations. RNA was then converted into a cDNA form using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). After this, cDNA from NPC1 carrier and wild-type mice was used for quantitative Real-Time PCR (qRT-PCR) using a LightCycler 480 SYBR Green I Master Mix chemistry (Roche Diagnostics, Indianapolis, IN) and analyzed on the LightCycler 480 instrument (Roche Diagnostics, Indianapolis,

IN). Expression levels of ATGL, HSL, MGL, LPL, CGI-58, PPARγ, Perilipin, Mal1, CD36, FATP1, FATP4, Ascl1, GOT2, and FAS. FATP4, CGI-58, MGL, GOT2, FAS, and ATGL genes were measured by a 3-step qtRT-PCR parameter with a pre-incubation step at 95℃ for 5 minutes, followed by amplification for 45 cycles at 95℃ for 10 seconds, 60℃ for 15 seconds, 72℃ for 1 second . HSL, Perilipin, CD36, PPARγ, Mal1, Ascl1, LPL, and FATP1 genes were run using a 2-step qtRT-PCR parameter with a pre-incubation step at 95℃ for 15 minutes, followed by amplification for 40 cycles at 95°C for 15 seconds, and at annealing temperature for 1 minute. Gene expression changes were calculated using a relative standard curve method (Livak, 1997) using 36B4 mRNA levels for normalization.

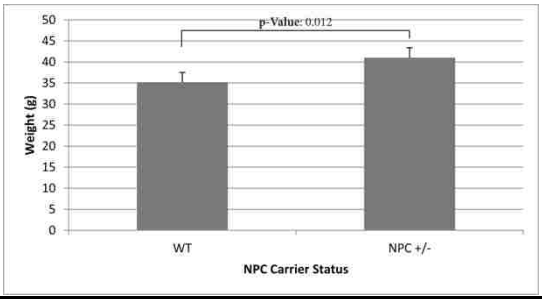
3.2.6 Statistical Analysis

Statistical significance was determined by performed t-tests of data in Microsoft Excel 2010. Statistical significance was determined if p-value < 0.05.

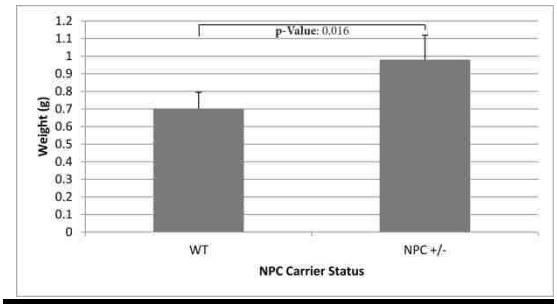
3.3 Results

3.3.1 NPC1 Carrier Weight Differential

The NPC1 gene variants have been previously associated with obesity in human models and weight gain in a carrier (+/-) mouse model. (Jelinek et al., 2012b; Jelinek, Heidenreich, Erickson, & Garver, 2009; Meyre, Delplanque, Chèvre, Lecoeur, Lobbens, Gallina, Durand, Vatin, Degraeve, Proença, et al., 2009c) Before assessing potential metabolic changes that may be occurring, it was again confirmed that a body weight differential was present between the NPC1 carrier and the wild-type mice (Figure-3.2). At age 160 days, body weight measurements were made. These measurements showed NPC1 carrier mice to be heavier with an average weight of 41.05g, while the wild-type mice were measured with an average weight closer to 35.2g. These differences were statistically significant.



To further establish the potential of a metabolic dysregulation, fat pad weight differential was also assessed. As expected, a significant weight differential existed between fat pads of the NPC1 carrier and wild-type mice (Figure-3.3). Average fat pad weight for NPC1 carrier mice was measured at 0.98g, while the wild-type mice had an average fat

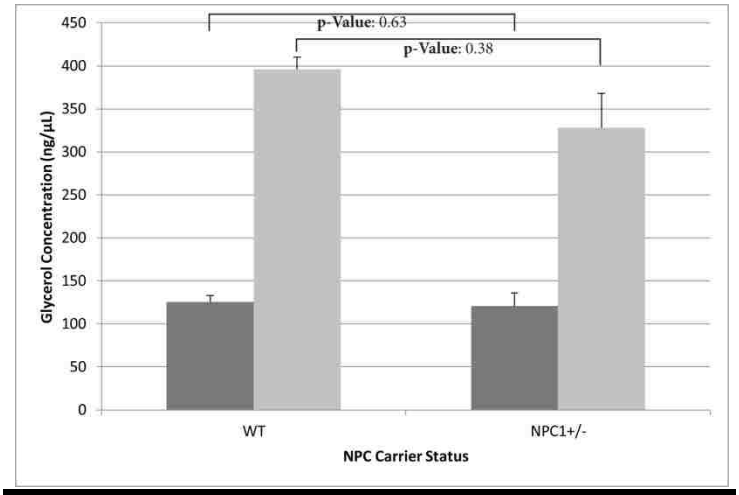


pad weight measured closer to 0.7g. Individual fat pad weights were used with the largest fat pad for either NPC1 carrier or wild-type being assessed.

3.3.2 NPC1 Carrier Glycerol Release Trend

As mentioned previously in chapter 2.2.2, glycerol release is a useful indicator of lipolytic function. (Aboulaich et al., 2011; Lamb et al., 2011; Schleich & Teleman, 2009) Again, following the enzymatic removal of all three fatty acids of a triglyceride, the glycerol backbone is freed and released into medium. By measuring the glycerol released into the medium, an overall assessment of lipolytic function can be determined. For these studies, a four hour time course was found to be sufficient to assess changes to lipolytic function. Because of β-adrenergic signaling being a normal physiological lipolytic stimulation response, this was the method used to assess a stimulated state in NPC1 carrier versus wild-type.

1μM isoproterenol was used as was previously established with 3T3-L1 adipocytes in chapter 2.3.1.



Basal level lipolytic activity was measured for both NPC1 carrier and wild-type mice and was found to be comparable (p-value of 0.63), the former having a 120.81ng/mL (standard deviation of

15.32) glycerol level and the later having a 125.3ng/mL (standard deviation of 7.74) glycerol level (Figure-3.4). However, when β-adrenergic stimulation was induced through 1μM isoproterenol treatment, a dramatic increase was shown, though NPC1 carrier mice demonstrated a slightly lower glycerol release level than that of the wild-type mice. Although these results are not statistically significant (p-value of 0.38), the reduced glycerol release by NPC1 carrier mice appears to demonstrate a decreased trend. The average glycerol release level for the NPC1 carrier mice was 327.95ng/mL (standard deviation of 39.94) glycerol while the wild-type was higher at 396.26ng/mL (standard deviation of 14.29).

3.3.3 Lipolytic and Lipogenic Genes

Lipolytic Genes		
Gene Name	% Difference	p-Value
HSL	**55.15**	**0.02**
Perilipin	**47.50**	**0.05**
ATGL	78.65	0.30
MGL	66.12	0.09
CGI-58	77.09	0.13
PPARγ	92.34	0.60
LPL	67.90	0.07

To further determine the impact of the NPC1 carrier phenotype on lipid metabolic function, a battery of genes were tested to determine if any trends or individual genes lead to the increased weight phenotype previously reported. (Jelinek, Heidenreich, Erickson, & Garver, 2010c) A variety of lipolytic, lipogenic, as well as transport proteins were assessed in comparison to the housekeeping gene 36B4. Lipolytic genes that were tested include HSL, ATGL, MGL, and LPL as

Lipogenic Genes		
Gene Name	% Difference	p-Value
FAS	48.74	0.06

well as lipolytic support proteins CGI-58, and perilipin (Table-3.1). Of these genes a significant decrease was observed in HSL mRNA level (Table-3.1), which was approximately 55% lower than the housekeeping gene. As mentioned previously, HSL is a key lipolytic gene whose expressed form is involved in triglyceride to diglyceride degradation. Another lipolytic gene that was observed to be decreased was perilipin, a lipid droplet surface protein, which was approximately 47% lower than the housekeeping gene (Table-3.1). (Lass, Zimmermann, Oberer, & Zechner, 2011b; Zechner, Kienesberger, Haemmerle, Zimmermann, & Lass, 2009f) In addition to lipolytic genes, FAS, a key lipogenic gene, was tested for. (Styer et al., 2011) FAS did not appear to be statistically significant in comparison to the housekeeping gene (Table-3.2). As mentioned in chapter 1.7, transport and translocation proteins are an important part of the fat metabolic processes, providing the means for fatty acids to enter and exit the cell as well as traverse highly hydrophilic environments. Transport and translocation genes that were tested for include CD36, FATP1, FATP4, Mal1, Ascl1, and GOT2 (Table-3.3). (Large et al., 2004b; Larqué et al., 2006; Maeda et al., 2003; Pelsers, Stellingzoerff, & Van Loon, 2008b; Stahl, 2004; Zhan, Poppelreuther, Ehehalt, & Füllekrug, 2012) Of these genes, Mal1/FABP5 was increased to approximately 134% of the housekeeping gene (Table-3.3).

Transport/Translocation		
Gene Name	% Difference	p-Value
FABP5/Mal1	**134.38**	**0.01**
CD36	102.41	0.88
FATP1	51.14	0.07
FATP4	80.17	0.29
Ascl1	84.55	0.39
FABPpm/GOT2	101.13	0.97

3.4 Conclusion

NPC1 gene variants have been associated with obesity in humans, as well as weight gain in NPC1 carrier mice when on a high-fat diet. (Jelinek, Heidenreich, Erickson, & Garver, 2010d; Meyre, Delplanque, Chèvre, Lecoeur, Lobbens, Gallina, Durand, Vatin, Degraeve, Proença, et al., 2009d) Our findings confirm a weight differential between NPC1 carrier mice and wild-type mice. This difference is present not only in whole body weight but also in fat pad weight. Information defining the impact of the NPC1 gene on metabolic regulation is presently lacking. To date, studies have only demonstrated that NPC1 participates in LDL based cholesterol trafficking from late endosomes/lysosomes to other cellular compartments. (Garver et al., 2000; Higgins et al., 1999; Jelinek et al., 2012a; Liscum et al., 1989)

To begin to define the impact NPC1 may have on lipolytic regulation and fat storage function, a series of experiments to assess glycerol release and fat metabolism gene levels was performed. In NPC1 carrier mice, glycerol release following beta-adrenergic stimulation was decreased, and although these data did not reach statistical significance, a strong trend was revealed. Including more mice in this assay will likely improve these findings. To further support that NPC1 may be involved in regulating lipid metabolic activities, a battery of real-time PCRs were performed on a variety of lipolytic, lipogenic, and transport/translocation genes. Of the genes that showed statistical significance, HSL is of particular interest because of its important role in the lipolytic process. (Lass, Zimmermann, Oberer, & Zechner, 2011c)

Experimental analysis provides some suggestion that decreased NPC1 gene dosage in haploinsufficient animals leads to increased fat pad weight by mechanisms that

decrease lipolytic genes. To further investigate this, additional studies examining HSL enzymatic activities are warranted.

Chapter 4: Overall Discussion:

4.1 Causes of Obesity Conclusion

Obesity is becoming a progressively important health concern in the United States as trends toward an increasingly obese population have been shown (data in chapter 1.2). ("Obesity and Overweight for Professionals: Data and Statistics: Adult Obesity - DNPAO - CDC," n.d.-b) Research has shown that obesity is generally caused by one or even a combination of three factors: environment, genetics, and social behaviors. (Nguyen & El-Serag, 2010b) Environmental factors can be generally attributed to one of two factors. In terms of energy intake in excess of energy expenditure; one of these factors is consuming more energy than one normally burns, and the other factor being expending less energy for a given food intake (also thought of as an increase in inactivity). (W H Dietz Jr & Gortmaker, 1985; Donahoo, Levine, & Melanson, 2004b; Kant & Graubard, 2006b; A. M. Prentice & Jebb, 1995) Either of these two factors has been shown to lead to obesity. In addition to environmental factors that have been shown to increase the risk of obesity, genetics has also been shown to increase this risk. Obesity-associated gene (FTO rs9939609) is a gene of particular interest for increasing the odds of obesity by 1.67-fold in patients who are homozygous for the gene. (Andreasen et al., 2008b; Frayling et al., 2007b; Hunt et al., 2008b) Another gene that has been shown to be associated with obesity is the NPC1 gene. (Meyre, Delplanque, Chèvre, Lecoeur, Lobbens, Gallina, Durand, Vatin, Degraeve, Proença, et al., 2009e) This data was supported in our findings in chapter 3.3.1 by a positive body weight and fat pad weight differential. Lastly, social behaviors have been linked with obesity in that personal associations, such as friends, siblings, spouses, etc., who are obese increase the risk of an

individual as well. Together these factors are all important to consider when addressing the increasing trend seen.

4.2 Effects (Direct Lipolytic Regulation)

Recent findings have led to the discovery that obese WAT demonstrates the presence of macrophage specific antigens as well as pro-inflammatory cytokines. (X.-H. Chen et al., 2010b; Galic, Oakhill, & Steinberg, 2010f; Jaworski, Sarkadi-Nagy, Duncan, Ahmadian, & Sul, 2007g; Ji et al., 2011d; Kershaw & Flier, 2004b; Langin & Arner, 2006b; Xu et al., 2003c; Zechner, Kienesberger, Haemmerle, Zimmermann, & Lass, 2009g) To better understand the lipolytic impact that compounding stimulating agents may have, we assessed a model system that includes independent stimulation of the pro-inflammatory cytokines TNFα and IL-6 as well as isoproterenol (a β-adrenergic agonist), co-stimulation of an individual cytokine with isoproterenol, as well as triple-stimulation with two cytokines and isoproterenol. Previous findings have demonstrated the impact of individual stimulating factors though the impact of multiple agents has not been examined. Our findings demonstrate that treatment of adipocytes with cytokines in combination with beta-adrenergic stimulation hasan additive effect on activating lipolytic activity. This finding clearly indicates that lipolytic stimulation in a complex obese biological system, where multiple agents are likely to be present, is much greater than simply assessing only a single agent system.

4.3 Effects (Indirect Lipolytic Regulation)

Lipolytic regulation as we currently understand it may be an oversimplified view when considering a complex obese biological systems. To better assess how a disease state may impact lipolytic regulation studying the impact of genetic causes for obesity is important as well. To investigate this, we examined lipid handling activity using a mouse NPC1 carrier model system. As mentioned previously NPC1 gene variants have been associated with obesity.(Meyre, Delplanque, Chèvre, Lecoeur, Lobbens, Gallina, Durand, Vatin, Degraeve, & Proen\cca, 2009) Our findings confirmed that increased body weight in the NPC1 carrier mice was at least partially due to a corresponding increase in fat pad weight (data shown in chapter 3.3.1). Beyond this we showed that there appears to be a trend of decreased glycerol release in the NPC1 carrier mice as well as decreased levels of HSL mRNA, a key lipolytic enzyme. These findings show that decreased NPC1 gene dosage, that is expected to impact cholesterol transport from the lysosome/late endosomes, may also have an indirect impact (NPC1 is not a lipolytic gene) on lipolytic regulation as well. (Meyre, Delplanque, Chèvre, Lecoeur, Lobbens, Gallina, Durand, Vatin, Degraeve, & Proença, 2009)

4.4 Compounding Effects of Lipolysis

In all, lipolytic regulation is an important component for proper fat metabolism. Many of the deleterious effects relating to fat metabolism appears to result from improper fat storage. In fact many findings have shown that lipotoxicity, the accumulation of fatty acids in non-adipose tissues, can lead to a variety of serious health concerns depending on the organ that begins to store them in. (Perez-Martinez, Perez-Jimenez, & Lopez-

Miranda, 2010b; Schaffer, 2003b; Unger, 2002b) Proper fat storage in adipocytes occurs through a series of biochemical pathways that include triglyceride synthesis, lipogenesis, transport and translocation of fatty acids, as well as lipolysis. Our findings demonstrate that compounding treatment of adipocytes with multiple lipolytic activators, as well as reduced gene dosage of NPC1, are capable of altering normal lipolytic regulation in adipocytes.

4.5 Future Studies

Further study can provide additional information on the signaling mechanisms involved in co- and triple-stimulation of the lipolytic processes. In addition, the findings reported here need to be confirmed and extended in an in vivo model of obesity, such as diet-induced obese mice. This will better represent a complex obese biological system and provide essential physiological data. Additionally, to better determine indirect mechanisms that effect lipolytic regulation, further study is needed to determine how a decrease in lipolytic genes, in combination with increased stimulation from pro-inflammatory cytokines, might impact a complex biological system.

References:

Aboulaich, N., Chui, P. C., Asara, J. M., Flier, J. S., & Maratos-Flier, E. (2011). Polymerase I and Transcript Release Factor Regulates Lipolysis via a Phosphorylation-Dependent Mechanism. *Diabetes*, *60*(3), 757–765. doi:10.2337/db10-0744

Abumrad, N., Harmon, C., & Ibrahimi, A. (1998). Membrane transport of long-chain fatty acids: evidence for a facilitated process. *Journal of Lipid Research*, *39*(12), 2309–2318.

Adipose tissue. In: Encyclopedia of Sports Medicine and Science - Google Scholar. (n.d.). Retrieved July 9, 2013, from http://scholar.google.com/scholar?q=Adipose+tissue.+In%3A+Encyclopedia+of+Sports+Medicine+and+Science&btnG=&hl=en&as_sdt=0%2C32

Andreasen, C. H., Stender-Petersen, K. L., Mogensen, M. S., Torekov, S. S., Wegner, L., Andersen, G., … Hansen, T. (2008a). Low Physical Activity Accentuates the Effect of the FTO rs9939609 Polymorphism on Body Fat Accumulation. *Diabetes*, *57*(1), 95–101. doi:10.2337/db07-0910

Andreasen, C. H., Stender-Petersen, K. L., Mogensen, M. S., Torekov, S. S., Wegner, L., Andersen, G., … Hansen, T. (2008b). Low Physical Activity Accentuates the Effect of the FTO rs9939609 Polymorphism on Body Fat Accumulation. *Diabetes*, *57*(1), 95–101. doi:10.2337/db07-0910

Anthonsen, M. W., Rönnstrand, L., Wernstedt, C., Degerman, E., & Holm, C. (1998). Identification of Novel Phosphorylation Sites in Hormone-sensitive Lipase That Are Phosphorylated in Response to Isoproterenol and Govern Activation Properties in Vitro. *Journal of Biological Chemistry*, *273*(1), 215–221. doi:10.1074/jbc.273.1.215

Assessing Your Weight and Health Risk. (n.d.-a). Retrieved July 9, 2013, from http://www.nhlbi.nih.gov/health/public/heart/obesity/lose_wt/risk.htm#limitations

Assessing Your Weight and Health Risk. (n.d.-b). Retrieved July 9, 2013, from http://www.nhlbi.nih.gov/health/public/heart/obesity/lose_wt/risk.htm

Bastard, J.-P., Jardel, C., Bruckert, E., Blondy, P., Capeau, J., Laville, M., … Hainque, B. (2000). Elevated Levels of Interleukin 6 Are Reduced in Serum and Subcutaneous Adipose Tissue of Obese Women after Weight Loss. *Journal of Clinical Endocrinology & Metabolism*, *85*(9), 3338–3342. doi:10.1210/jc.85.9.3338

Bastard, J.-P., Maachi, M., Nhieu, J. T. van, Jardel, C., Bruckert, E., Grimaldi, A., … Hainque, B. (2002). Adipose Tissue IL-6 Content Correlates with Resistance to Insulin Activation of Glucose Uptake both in Vivo and in Vitro. *Journal of Clinical Endocrinology & Metabolism*, *87*(5), 2084–2089. doi:10.1210/jc.87.5.2084

Bertrand, H. A., Masoro, E. J., & YU, B. P. (1980). Maintenance of glucagon-promoted lipolysis in adipocytes by food restriction. *Endocrinology*, *107*(2), 591–595.

Bray, G. A., & Popkin, B. M. (1998). Dietary fat intake does affect obesity! *The American journal of clinical nutrition*, *68*(6), 1157–1173.

Brubaker, P. L., & Drucker, D. J. (2002). Structure-function of the glucagon receptor family of G protein-coupled receptors: the glucagon, GIP, GLP-1, and GLP-2 receptors. *Receptors and Channels*, *8*(3-4), 179–188.

Chaves, V. E., Frasson, D., & Kawashita, N. H. (2011a). Several agents and pathways regulate lipolysis in adipocytes. *Biochimie*, *93*(10), 1631–1640.

Chaves, V. E., Frasson, D., & Kawashita, N. H. (2011b). Several agents and pathways regulate lipolysis in adipocytes. *Biochimie*, *93*(10), 1631–1640.

Chen, X., Xun, K., Chen, L., & Wang, Y. (2009a). TNF-α, a potent lipid metabolism regulator. *Cell Biochemistry and Function*, *27*(7), 407–416. doi:10.1002/cbf.1596

Chen, X., Xun, K., Chen, L., & Wang, Y. (2009b). TNF-alpha, a potent lipid metabolism regulator. *Cell biochemistry and function*, *27*(7), 407–416. doi:10.1002/cbf.1596

Chen, X.-H., Zhao, Y.-P., Xue, M., Ji, C.-B., Gao, C.-L., Zhu, J.-G., … Tong, M.-L. (2010a). TNF-α induces mitochondrial dysfunction in 3T3-L1 adipocytes. *Molecular and cellular endocrinology*, *328*(1), 63–69.

Chen, X.-H., Zhao, Y.-P., Xue, M., Ji, C.-B., Gao, C.-L., Zhu, J.-G., … Tong, M.-L. (2010b). TNF-α induces mitochondrial dysfunction in 3T3-L1 adipocytes. *Molecular and cellular endocrinology*, *328*(1), 63–69.

Clark, H. R., Goyder, E., Bissell, P., Blank, L., & Peters, J. (2007). How do parents' child-feeding behaviours influence child weight? Implications for childhood obesity policy. *Journal of public health (Oxford, England)*, *29*(2), 132–141. doi:10.1093/pubmed/fdm012

Craig, B. W., Garthwaite, S. M., & Holloszy, J. O. (1987). Adipocyte insulin resistance: effects of aging, obesity, exercise, and food restriction. *Journal of applied physiology (Bethesda, Md.: 1985)*, *62*(1), 95–100.

Degerman, E., Landström, T. R., Wijkander, J., Holst, L. S., Ahmad, F., Belfrage, P., & Manganiello, V. (1998). Phosphorylation and Activation of Hormone-Sensitive Adipocyte Phosphodiesterase Type 3B. *Methods*, *14*(1), 43–53. doi:10.1006/meth.1997.0564

Dietz, W H, Jr, & Gortmaker, S. L. (1985). Do we fatten our children at the television set? Obesity and television viewing in children and adolescents. *Pediatrics*, *75*(5), 807–812.

Dietz, William H., & Gortmaker, S. L. (1985). Do We Fatten Our Children at the Television Set? Obesity and Television Viewing in Children and Adolescents. *Pediatrics*, *75*(5), 807–812.

Donahoo, W. T., Levine, J. A., & Melanson, E. L. (2004a). Variability in energy expenditure and its components. *Current Opinion in Clinical Nutrition & Metabolic Care*, *7*(6), 599–605.

Donahoo, W. T., Levine, J. A., & Melanson, E. L. (2004b). Variability in energy expenditure and its components. *Current Opinion in Clinical Nutrition & Metabolic Care*, *7*(6), 599–605.

Eknoyan, G. (2008). Adolphe Quetelet (1796–1874)—the average man and indices of obesity. *Nephrology Dialysis Transplantation*, *23*(1), 47–51. doi:10.1093/ndt/gfm517

Feingold, K. R., Doerrler, W., Dinarello, C. A., Fiers, W., & Grunfeld, C. (1992a). Stimulation of lipolysis in cultured fat cells by tumor necrosis factor, interleukin-1, and the interferons is blocked by inhibition of prostaglandin synthesis. *Endocrinology*, *130*(1), 10–16.

Feingold, K. R., Doerrler, W., Dinarello, C. A., Fiers, W., & Grunfeld, C. (1992b). Stimulation of lipolysis in cultured fat cells by tumor necrosis factor, interleukin-1, and the interferons is blocked by inhibition of prostaglandin synthesis. *Endocrinology*, *130*(1), 10–16.

Foufelle, F., & Ferré, P. (2002). New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *Biochemical Journal*, *366*(2), 377. doi:10.1042/BJ20020430

Frayling, T. M., Timpson, N. J., Weedon, M. N., Zeggini, E., Freathy, R. M., Lindgren, C. M., … McCarthy, M. I. (2007a). A Common Variant in the FTO Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. *Science*, *316*(5826), 889–894. doi:10.1126/science.1141634

Frayling, T. M., Timpson, N. J., Weedon, M. N., Zeggini, E., Freathy, R. M., Lindgren, C. M., … McCarthy, M. I. (2007b). A Common Variant in the FTO Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. *Science (New York, N.Y.)*, *316*(5826), 889–894. doi:10.1126/science.1141634

Fried, S. K., Bunkin, D. A., & Greenberg, A. S. (1998). Omental and Subcutaneous Adipose Tissues of Obese Subjects Release Interleukin-6: Depot Difference and Regulation by

Glucocorticoid. *Journal of Clinical Endocrinology & Metabolism*, *83*(3), 847–850. doi:10.1210/jc.83.3.847

Gaesser, G. A. (2007). Carbohydrate Quantity and Quality in Relation to Body Mass Index. *Journal of the American Dietetic Association*, *107*(10), 1768–1780. doi:10.1016/j.jada.2007.07.011

Galic, S., Oakhill, J. S., & Steinberg, G. R. (2010a). Adipose tissue as an endocrine organ. *Molecular and cellular endocrinology*, *316*(2), 129–139.

Galic, S., Oakhill, J. S., & Steinberg, G. R. (2010b). Adipose tissue as an endocrine organ. *Molecular and cellular endocrinology*, *316*(2), 129–139.

Galic, S., Oakhill, J. S., & Steinberg, G. R. (2010c). Adipose tissue as an endocrine organ. *Molecular and cellular endocrinology*, *316*(2), 129–139.

Galic, S., Oakhill, J. S., & Steinberg, G. R. (2010d). Adipose tissue as an endocrine organ. *Molecular and Cellular Endocrinology*, *316*(2), 129–139. doi:10.1016/j.mce.2009.08.018

Galic, S., Oakhill, J. S., & Steinberg, G. R. (2010e). Adipose tissue as an endocrine organ. *Molecular and Cellular Endocrinology*, *316*(2), 129–139. doi:10.1016/j.mce.2009.08.018

Galic, S., Oakhill, J. S., & Steinberg, G. R. (2010f). Adipose tissue as an endocrine organ. *Molecular and cellular endocrinology*, *316*(2), 129–139.

Garver, W. S., Heidenreich, R. A., Erickson, R. P., Thomas, M. A., & Wilson, J. M. (2000). Localization of the murine Niemann-Pick C1 protein to two distinct intracellular compartments. *Journal of Lipid Research*, *41*(5), 673–687.

Glueck, C. J., Fontaine, R. N., Wang, P., Subbiah, M. T., Weber, K., Illig, E., … McCullough, P. (2001). Metformin reduces weight, centripetal obesity, insulin, leptin, and low-density lipoprotein cholesterol in nondiabetic, morbidly obese subjects with body mass index

greater than 30. *Metabolism: clinical and experimental*, *50*(7), 856–861. doi:10.1053/meta.2001.24192

Hankey, C. R., Eley, S., Leslie, W. S., Hunter, C. M., & Lean, M. E. J. (2004). Eating habits, beliefs, attitudes and knowledge among health professionals regarding the links between obesity, nutrition and health. *Public health nutrition*, *7*(2), 337–343. doi:10.1079/PHN2003526

Healthy Weight: Assessing Your Weight: Body Mass Index (BMI) | DNPAO | CDC. (n.d.). Retrieved July 9, 2013, from http://www.cdc.gov/healthyweight/assessing/bmi/

HECKEMEYER, C. M., BARKER, J., DUCKWORTH, W. C., & SOLOMON, S. S. (1983). Studies of the biological effect and degradation of glucagon in the rat perifused isolated adipose cell. *Endocrinology*, *113*(1), 270–276.

Hems, D. A., Rath, E. A., & Verrinder, T. R. (1975). Fatty acid synthesis in liver and adipose tissue of normal and genetically obese (ob/ob) mice during the 24-hour cycle. *Biochemical Journal*, *150*(2), 167–173.

Higgins, M. E., Davies, J. P., Chen, F. W., & Ioannou, Y. A. (1999). Niemann–Pick C1 is a late endosome-resident protein that transiently associates with lysosomes and the trans-Golgi network. *Molecular genetics and metabolism*, *68*(1), 1–13.

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*, *259*(5091), 87–91.

Hunt, S. C., Stone, S., Xin, Y., Scherer, C. A., Magness, C. L., Iadonato, S. P., … Adams, T. D. (2008a). Association of the FTO Gene With BMI. *Obesity*, *16*(4), 902–904. doi:10.1038/oby.2007.126

Hunt, S. C., Stone, S., Xin, Y., Scherer, C. A., Magness, C. L., Iadonato, S. P., … Adams, T. D. (2008b). Association of the FTO gene with BMI. *Obesity (Silver Spring, Md.)*, *16*(4), 902–904. doi:10.1038/oby.2007.126

Jaime, P. C., & Lock, K. (2009). Do school based food and nutrition policies improve diet and reduce obesity? *Preventive medicine*, *48*(1), 45–53. doi:10.1016/j.ypmed.2008.10.018

Jaworski, K., Sarkadi-Nagy, E., Duncan, R. E., Ahmadian, M., & Sul, H. S. (2007a). Regulation of Triglyceride Metabolism.IV. Hormonal regulation of lipolysis in adipose tissue. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *293*(1), G1–G4. doi:10.1152/ajpgi.00554.2006

Jaworski, K., Sarkadi-Nagy, E., Duncan, R. E., Ahmadian, M., & Sul, H. S. (2007b). Regulation of Triglyceride Metabolism.IV. Hormonal regulation of lipolysis in adipose tissue. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *293*(1), G1–G4. doi:10.1152/ajpgi.00554.2006

Jaworski, K., Sarkadi-Nagy, E., Duncan, R. E., Ahmadian, M., & Sul, H. S. (2007c). Regulation of Triglyceride Metabolism.IV. Hormonal regulation of lipolysis in adipose tissue. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *293*(1), G1–G4. doi:10.1152/ajpgi.00554.2006

Jaworski, K., Sarkadi-Nagy, E., Duncan, R. E., Ahmadian, M., & Sul, H. S. (2007d). Regulation of Triglyceride Metabolism.IV. Hormonal regulation of lipolysis in adipose tissue. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *293*(1), G1–G4. doi:10.1152/ajpgi.00554.2006

Jaworski, K., Sarkadi-Nagy, E., Duncan, R. E., Ahmadian, M., & Sul, H. S. (2007e). Regulation of Triglyceride Metabolism. IV. Hormonal regulation of lipolysis in adipose tissue. *American*

journal of physiology. Gastrointestinal and liver physiology, *293*(1), G1–G4. doi:10.1152/ajpgi.00554.2006

Jaworski, K., Sarkadi-Nagy, E., Duncan, R. E., Ahmadian, M., & Sul, H. S. (2007f). Regulation of Triglyceride Metabolism.IV. Hormonal regulation of lipolysis in adipose tissue. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *293*(1), G1–G4. doi:10.1152/ajpgi.00554.2006

Jaworski, K., Sarkadi-Nagy, E., Duncan, R. E., Ahmadian, M., & Sul, H. S. (2007g). Regulation of Triglyceride Metabolism. IV. Hormonal regulation of lipolysis in adipose tissue. *American journal of physiology. Gastrointestinal and liver physiology*, *293*(1), G1–G4. doi:10.1152/ajpgi.00554.2006

Jelinek, D., Castillo, J. J., Richardson, L. M., Luo, L., Heidenreich, R. A., & Garver, W. S. (2012a). The Niemann-Pick C1 gene is downregulated in livers of C57BL/6J mice by dietary fatty acids, but not dietary cholesterol, through feedback inhibition of the SREBP pathway. *The Journal of nutrition*, *142*(11), 1935–1942.

Jelinek, D., Castillo, J. J., Richardson, L. M., Luo, L., Heidenreich, R. A., & Garver, W. S. (2012b). The Niemann-Pick C1 Gene Is Downregulated in Livers of C57BL/6J Mice by Dietary Fatty Acids, but Not Dietary Cholesterol, through Feedback Inhibition of the SREBP Pathway. *The Journal of Nutrition*, *142*(11), 1935–1942. doi:10.3945/jn.112.162818

Jelinek, D., Heidenreich, R. A., Erickson, R. P., & Garver, W. S. (2009). Decreased Npc1 Gene Dosage in Mice Is Associated With Weight Gain. *Obesity*, *18*(7), 1457–1459. doi:10.1038/oby.2009.415

Jelinek, D., Heidenreich, R. A., Erickson, R. P., & Garver, W. S. (2010a). Decreased Npc1 Gene Dosage in Mice Is Associated With Weight Gain. *Obesity (Silver Spring, Md.)*, *18*(7), 1457–1459. doi:10.1038/oby.2009.415

Jelinek, D., Heidenreich, R. A., Erickson, R. P., & Garver, W. S. (2010b). Decreased Npc1 Gene Dosage in Mice Is Associated With Weight Gain. *Obesity (Silver Spring, Md.)*, *18*(7), 1457–1459. doi:10.1038/oby.2009.415

Jelinek, D., Heidenreich, R. A., Erickson, R. P., & Garver, W. S. (2010c). Decreased Npc1 gene dosage in mice is associated with weight gain. *Obesity (Silver Spring, Md.)*, *18*(7), 1457–1459. doi:10.1038/oby.2009.415

Jelinek, D., Heidenreich, R. A., Erickson, R. P., & Garver, W. S. (2010d). Decreased Npc1 gene dosage in mice is associated with weight gain. *Obesity (Silver Spring, Md.)*, *18*(7), 1457–1459. doi:10.1038/oby.2009.415

Ji, C., Chen, X., Gao, C., Jiao, L., Wang, J., Xu, G., … Zhao, Y. (2011a). IL-6 induces lipolysis and mitochondrial dysfunction, but does not affect insulin-mediated glucose transport in 3T3-L1 adipocytes. *Journal of bioenergetics and biomembranes*, *43*(4), 367–375.

Ji, C., Chen, X., Gao, C., Jiao, L., Wang, J., Xu, G., … Zhao, Y. (2011b). IL-6 induces lipolysis and mitochondrial dysfunction, but does not affect insulin-mediated glucose transport in 3T3-L1 adipocytes. *Journal of Bioenergetics and Biomembranes*, *43*(4), 367–375. doi:10.1007/s10863-011-9361-8

Ji, C., Chen, X., Gao, C., Jiao, L., Wang, J., Xu, G., … Zhao, Y. (2011c). IL-6 induces lipolysis and mitochondrial dysfunction, but does not affect insulin-mediated glucose transport in 3T3-L1 adipocytes. *Journal of bioenergetics and biomembranes*, *43*(4), 367–375. doi:10.1007/s10863-011-9361-8

Ji, C., Chen, X., Gao, C., Jiao, L., Wang, J., Xu, G., … Zhao, Y. (2011d). IL-6 induces lipolysis and mitochondrial dysfunction, but does not affect insulin-mediated glucose transport in 3T3-L1 adipocytes. *Journal of bioenergetics and biomembranes*, *43*(4), 367–375.

Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K., Kitazawa, R., … Kasuga, M. (2006). MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *Journal of Clinical Investigation*, *116*(6), 1494–1505. doi:10.1172/JCI26498

Kant, A. K., & Graubard, B. I. (2006a). Secular trends in patterns of self-reported food consumption of adult Americans: NHANES 1971-1975 to NHANES 1999–2002. *The American Journal of Clinical Nutrition*, *84*(5), 1215–1223.

Kant, A. K., & Graubard, B. I. (2006b). Secular trends in patterns of self-reported food consumption of adult Americans: NHANES 1971-1975 to NHANES 1999-2002. *The American journal of clinical nutrition*, *84*(5), 1215–1223.

Kershaw, E. E., & Flier, J. S. (2004a). Adipose Tissue as an Endocrine Organ. *Journal of Clinical Endocrinology & Metabolism*, *89*(6), 2548–2556. doi:10.1210/jc.2004-0395

Kershaw, E. E., & Flier, J. S. (2004b). Adipose Tissue as an Endocrine Organ. *Journal of Clinical Endocrinology & Metabolism*, *89*(6), 2548–2556. doi:10.1210/jc.2004-0395

Koo, S.-H., Dutcher, A. K., & Towle, H. C. (2001). Glucose and Insulin Function through Two Distinct Transcription Factors to Stimulate Expression of Lipogenic Enzyme Genes in Liver. *Journal of Biological Chemistry*, *276*(12), 9437–9445. doi:10.1074/jbc.M010029200

Lamb, T. M., Goldsmith, C. S., Bennett, L., Finch, K. E., & Bell-Pedersen, D. (2011). Direct Transcriptional Control of a p38 MAPK Pathway by the Circadian Clock in Neurospora crassa. *PLoS ONE*, *6*(11). doi:10.1371/journal.pone.0027149

Langin, D. (2006a). Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. *Pharmacological Research*, *53*(6), 482–491.

Langin, D. (2006b). Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. *Pharmacological research: the official journal of the Italian Pharmacological Society*, *53*(6), 482–491. doi:10.1016/j.phrs.2006.03.009

Langin, D., & Arner, P. (2006a). Importance of TNFα and neutral lipases in human adipose tissue lipolysis. *Trends in Endocrinology & Metabolism*, *17*(8), 314–320. doi:10.1016/j.tem.2006.08.003

Langin, D., & Arner, P. (2006b). Importance of TNFα and neutral lipases in human adipose tissue lipolysis. *Trends in Endocrinology & Metabolism*, *17*(8), 314–320. doi:10.1016/j.tem.2006.08.003

Large, V., Peroni, O., Letexier, D., Ray, H., & Beylot, M. (2004a). Metabolism of lipids in human white adipocyte. *Diabetes & Metabolism*, *30*(4), 294–309. doi:10.1016/S1262-3636(07)70121-0

Large, V., Peroni, O., Letexier, D., Ray, H., & Beylot, M. (2004b). Metabolism of lipids in human white adipocyte. *Diabetes & Metabolism*, *30*(4), 294–309. doi:10.1016/S1262-3636(07)70121-0

Large, V., Peroni, O., Letexier, D., Ray, H., & Beylot, M. (2004c). Metabolism of lipids in human white adipocyte. *Diabetes & Metabolism*, *30*(4), 294–309. doi:10.1016/S1262-3636(07)70121-0

Large, V., Peroni, O., Letexier, D., Ray, H., & Beylot, M. (2004d). Metabolism of lipids in human white adipocyte. *Diabetes & Metabolism*, *30*(4), 294–309. doi:10.1016/S1262-3636(07)70121-0

Large, V., Peroni, O., Letexier, D., Ray, H., & Beylot, M. (2004e). Metabolism of lipids in human white adipocyte. *Diabetes & metabolism*, *30*(4), 294–309.

Large, V., Peroni, O., Letexier, D., Ray, H., & Beylot, M. (2004f). Metabolism of lipids in human white adipocyte. *Diabetes & metabolism*, *30*(4), 294–309.

Larqué, E., Krauss-Etschmann, S., Campoy, C., Hartl, D., Linde, J., Klingler, M., … Koletzko, B. (2006). Docosahexaenoic acid supply in pregnancy affects placental expression of fatty acid transport proteins. *The American Journal of Clinical Nutrition*, *84*(4), 853–861.

Lass, A., Zimmermann, R., Oberer, M., & Zechner, R. (2011a). Lipolysis – A highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores. *Progress in Lipid Research*, *50*(1), 14–27. doi:10.1016/j.plipres.2010.10.004

Lass, A., Zimmermann, R., Oberer, M., & Zechner, R. (2011b). Lipolysis – A highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores. *Progress in Lipid Research*, *50*(1), 14–27. doi:10.1016/j.plipres.2010.10.004

Lass, A., Zimmermann, R., Oberer, M., & Zechner, R. (2011c). Lipolysis - a highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores. *Progress in lipid research*, *50*(1), 14–27. doi:10.1016/j.plipres.2010.10.004

Liscum, L., Ruggiero, R. M., & Faust, J. R. (1989). The intracellular transport of low density lipoprotein-derived cholesterol is defective in Niemann-Pick type C fibroblasts. *The Journal of Cell Biology*, *108*(5), 1625–1636. doi:10.1083/jcb.108.5.1625

Lissner, L., & Heitmann, B. L. (1995). Dietary fat and obesity: evidence from epidemiology. *European journal of clinical nutrition*, *49*(2), 79–90.

Livak, K. J. (1997). ABI Prism 7700 sequence detection system. *User bulletin*, *2*, 1–36.

Maeda, K., Uysal, K. T., Makowski, L., Görgün, C. Z., Atsumi, G., Parker, R. A., … Hotamisligil, G. S. (2003). Role of the Fatty Acid Binding Protein mal1 in Obesity and Insulin Resistance. *Diabetes*, *52*(2), 300–307. doi:10.2337/diabetes.52.2.300

Meyre, D., Delplanque, J., Chèvre, J.-C., Lecoeur, C., Lobbens, S., Gallina, S., … Froguel, P. (2009a). Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nature Genetics*, *41*(2), 157–159. doi:10.1038/ng.301

Meyre, D., Delplanque, J., Chèvre, J.-C., Lecoeur, C., Lobbens, S., Gallina, S., … Froguel, P. (2009b). Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nature Genetics*, *41*(2), 157–159. doi:10.1038/ng.301

Meyre, D., Delplanque, J., Chèvre, J.-C., Lecoeur, C., Lobbens, S., Gallina, S., … Froguel, P. (2009c). Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nature Genetics*, *41*(2), 157–159. doi:10.1038/ng.301

Meyre, D., Delplanque, J., Chèvre, J.-C., Lecoeur, C., Lobbens, S., Gallina, S., … Froguel, P. (2009d). Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nature genetics*, *41*(2), 157–159. doi:10.1038/ng.301

Meyre, D., Delplanque, J., Chèvre, J.-C., Lecoeur, C., Lobbens, S., Gallina, S., … Froguel, P. (2009e). Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nature Genetics*, *41*(2), 157–159. doi:10.1038/ng.301

Meyre, D., Delplanque, J., Chèvre, J.-C., Lecoeur, C., Lobbens, S., Gallina, S., … Proença, C. (2009). Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nature genetics*, *41*(2), 157–159.

Mokdad AH, F. E. (2003). PRevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA*, *289*(1), 76–79. doi:10.1001/jama.289.1.76

Murphy, S., Martin, S., & Parton, R. G. (2009). Lipid droplet-organelle interactions; sharing the fats. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1791*(6), 441–447. doi:10.1016/j.bbalip.2008.07.004

Nguyen, D. M., & El-Serag, H. B. (2010a). The Epidemiology of Obesity. *Gastroenterology Clinics of North America*, *39*(1), 1–7. doi:10.1016/j.gtc.2009.12.014

Nguyen, D. M., & El-Serag, H. B. (2010b). The Epidemiology of Obesity. *Gastroenterology clinics of North America*, *39*(1), 1–7. doi:10.1016/j.gtc.2009.12.014

Niemann-Pick disease - National Library of Medicine - PubMed Health. (n.d.-a). Retrieved July 9, 2013, from http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0002187/

Niemann-Pick disease - National Library of Medicine - PubMed Health. (n.d.-b). Retrieved July 9, 2013, from http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0002187/

Nieto-Vazquez, I., Fernández-Veledo, S., Krämer, D. K., Vila-Bedmar, R., Garcia-Guerra, L., & Lorenzo, M. (2008). Insulin resistance associated to obesity: the link TNF-alpha. *Archives Of Physiology And Biochemistry*, *114*(3), 183–194. doi:10.1080/13813450802181047

NIH Categorical Spending -NIH Research Portfolio Online Reporting Tools (RePORT). (n.d.). Retrieved July 9, 2013, from http://report.nih.gov/categorical_spending.aspx

Obesity and Overweight for Professionals: Adult: Defining - DNPAO - CDC. (n.d.). Retrieved July 9, 2013, from http://www.cdc.gov/obesity/adult/defining.html

Obesity and Overweight for Professionals: Data and Statistics: Adult Obesity - DNPAO - CDC. (n.d.-a). Retrieved July 9, 2013, from http://www.cdc.gov/obesity/data/adult.html

Obesity and Overweight for Professionals: Data and Statistics: Adult Obesity - DNPAO - CDC. (n.d.-b). Retrieved July 10, 2013, from http://www.cdc.gov/obesity/data/adult.html

Obesity: MedlinePlus. (n.d.). Retrieved July 9, 2013, from http://www.nlm.nih.gov/medlineplus/obesity.html

Olofsson, S.-O., Boström, P., Andersson, L., Rutberg, M., Levin, M., Perman, J., & Borén, J. (2008). Triglyceride containing lipid droplets and lipid droplet-associated proteins. *Current Opinion in Lipidology*, *19*(5), 441–447. doi:10.1097/MOL.0b013e32830dd09b

Patterson, M. C., Hendriksz, C. J., Walterfang, M., Sedel, F., Vanier, M. T., & Wijburg, F. (2012a). Recommendations for the diagnosis and management of Niemann–Pick disease type C: An update. *Molecular genetics and metabolism*, *106*(3), 330–344.

Patterson, M. C., Hendriksz, C. J., Walterfang, M., Sedel, F., Vanier, M. T., & Wijburg, F. (2012b). Recommendations for the diagnosis and management of Niemann–Pick disease type C: An update. *Molecular genetics and metabolism*, *106*(3), 330–344.

Pelsers, M. M. A. L., Stellingzoerff, T., & Van Loon, L. J. C. (2008a). The Role of Membrane Fatty-Acid Transporters in Regulating Skeletal Muscle Substrate Use during Exercise. *Sports Medicine*, *38*(5), 387–399.

Pelsers, M. M. A. L., Stellingzoerff, T., & Van Loon, L. J. C. (2008b). The Role of Membrane Fatty-Acid Transporters in Regulating Skeletal Muscle Substrate Use during Exercise. *Sports Medicine*, *38*(5), 387–399.

Perez-Martinez, P., Perez-Jimenez, F., & Lopez-Miranda, J. (2010a). n-3 PUFA and lipotoxicity. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1801*(3), 362–366. doi:10.1016/j.bbalip.2009.09.010

Perez-Martinez, P., Perez-Jimenez, F., & Lopez-Miranda, J. (2010b). < i> n</i>-3 PUFA and lipotoxicity. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1801*(3), 362–366.

Perreault, M., & Marette, A. (2001). Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nature medicine*, *7*(10), 1138–1143.

Popkin, B. M., & Gordon-Larsen, P. (2004). The nutrition transition: worldwide obesity dynamics and their determinants. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity*, *28 Suppl 3*, S2–9. doi:10.1038/sj.ijo.0802804

Preiss-Landl, K., Zimmermann, R., Hämmerle, G., & Zechner, R. (2002). Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. *Current opinion in lipidology*, *13*(5), 471–481.

Prentice, A. M., & Jebb, S. A. (1995). Obesity in Britain: gluttony or sloth? *BMJ : British Medical Journal*, *311*(7002), 437–439.

Prentice, Andrew M., & Jebb, S. A. (1995). Obesity in Britain: gluttony or sloth? *BMJ: British Medical Journal*, *311*(7002), 437.

Rankinen, T., Zuberi, A., Chagnon, Y. C., Weisnagel, S. J., Argyropoulos, G., Walts, B., … Bouchard, C. (2006). The Human Obesity Gene Map: The 2005 Update. *Obesity*, *14*(4), 529–644. doi:10.1038/oby.2006.71

Riccardi, G., Giacco, R., & Rivellese, A. . (2004). Dietary fat, insulin sensitivity and the metabolic syndrome. *Clinical Nutrition*, *23*(4), 447–456. doi:10.1016/j.clnu.2004.02.006

Samad, F., Yamamoto, K., Pandey, M., & Loskutoff, D. J. (1997). Elevated expression of transforming growth factor-beta in adipose tissue from obese mice. *Molecular Medicine*, *3*(1), 37–48.

Sartipy, P., & Loskutoff, D. J. (2003). Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(12), 7265–7270. doi:10.1073/pnas.1133870100

Schaffer, J. E. (2003a). Lipotoxicity: when tissues overeat. *Current opinion in lipidology*, *14*(3), 281–287.

Schaffer, J. E. (2003b). Lipotoxicity: when tissues overeat. *Current opinion in lipidology*, *14*(3), 281–287.

Schleich, S., & Teleman, A. A. (2009). Akt Phosphorylates Both Tsc1 and Tsc2 in Drosophila, but Neither Phosphorylation Is Required for Normal Animal Growth. *PLoS ONE*, *4*(7). doi:10.1371/journal.pone.0006305

Souza, S C, de Vargas, L. M., Yamamoto, M. T., Lien, P., Franciosa, M. D., Moss, L. G., & Greenberg, A. S. (1998). Overexpression of perilipin A and B blocks the ability of tumor necrosis factor alpha to increase lipolysis in 3T3-L1 adipocytes. *The Journal of biological chemistry*, *273*(38), 24665–24669.

Souza, Sandra C., Vargas, L. M. de, Yamamoto, M. T., Lien, P., Franciosa, M. D., Moss, L. G., & Greenberg, A. S. (1998). Overexpression of Perilipin A and B Blocks the Ability of Tumor Necrosis Factor α to Increase Lipolysis in 3T3-L1 Adipocytes. *Journal of Biological Chemistry*, *273*(38), 24665–24669. doi:10.1074/jbc.273.38.24665

Stahl, A. (2004). A current review of fatty acid transport proteins (SLC27). *Pflügers Archiv*, *447*(5), 722–727. doi:10.1007/s00424-003-1106-z

Styer, L., Tymoczko, J., & Berg, J. (2011). *Biochemistry: a Short Course.* W H Freeman & Co.

Subramanian, V., Rothenberg, A., Gomez, C., Cohen, A. W., Garcia, A., Bhattacharyya, S., … Brasaemle, D. L. (2004a). Perilipin A Mediates the Reversible Binding of CGI-58 to Lipid

Droplets in 3T3-L1 Adipocytes. *Journal of Biological Chemistry*, *279*(40), 42062–42071. doi:10.1074/jbc.M407462200

Subramanian, V., Rothenberg, A., Gomez, C., Cohen, A. W., Garcia, A., Bhattacharyya, S., … Brasaemle, D. L. (2004b). Perilipin A Mediates the Reversible Binding of CGI-58 to Lipid Droplets in 3T3-L1 Adipocytes. *Journal of Biological Chemistry*, *279*(40), 42062–42071. doi:10.1074/jbc.M407462200

Subramanian, V., Rothenberg, A., Gomez, C., Cohen, A. W., Garcia, A., Bhattacharyya, S., … Brasaemle, D. L. (2004c). Perilipin A mediates the reversible binding of CGI-58 to lipid droplets in 3T3-L1 adipocytes. *The Journal of biological chemistry*, *279*(40), 42062–42071. doi:10.1074/jbc.M407462200

Unger, R. H. (2002a). Lipotoxic Diseases. *Annual Review of Medicine*, *53*(1), 319–336. doi:10.1146/annurev.med.53.082901.104057

Unger, R. H. (2002b). Lipotoxic Diseases. *Annual Review of Medicine*, *53*(1), 319–336. doi:10.1146/annurev.med.53.082901.104057

Van Dam, R. M., & Seidell, J. C. (2007). Carbohydrate intake and obesity. *European Journal of Clinical Nutrition*, *61*(S1), S75–S99. doi:10.1038/sj.ejcn.1602939

Vanier, M. T. (2010). Niemann-Pick disease type C. *Orphanet Journal of Rare Diseases*, *5*, 16. doi:10.1186/1750-1172-5-16

Vgontzas, A. N., Papanicolaou, D. A., Bixler, E. O., Kales, A., Tyson, K., & Chrousos, G. P. (1997). Elevation of Plasma Cytokines in Disorders of Excessive Daytime Sleepiness: Role of Sleep Disturbance and Obesity. *Journal of Clinical Endocrinology & Metabolism*, *82*(5), 1313–1316. doi:10.1210/jc.82.5.1313

Visser M, B. L. (1999). ELevated c-reactive protein levels in overweight and obese adults. *JAMA*, *282*(22), 2131–2135. doi:10.1001/jama.282.22.2131

Wang, H., Bell, M., Sreenevasan, U., Hu, H., Liu, J., Dalen, K., … Sztalryd, C. (2011). Unique Regulation of Adipose Triglyceride Lipase (ATGL) by Perilipin 5, a Lipid Droplet-associated Protein. *Journal of Biological Chemistry*, *286*(18), 15707–15715. doi:10.1074/jbc.M110.207779

Wang, H., & Eckel, R. H. (2009). Lipoprotein lipase: from gene to obesity. *American Journal of Physiology - Endocrinology And Metabolism*, *297*(2), E271–E288. doi:10.1152/ajpendo.90920.2008

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003a). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, *112*(12), 1796–1808. doi:10.1172/JCI200319246

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003b). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, *112*(12), 1796–1808. doi:10.1172/JCI200319246

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W., Jr. (2003c). Obesity is associated with macrophage accumulation in adipose tissue. *The Journal of clinical investigation*, *112*(12), 1796–1808. doi:10.1172/JCI19246

Wellen, K. E., & Hotamisligil, G. S. (2003). Obesity-induced inflammatory changes in adipose tissue. *Journal of Clinical Investigation*, *112*(12), 1785–1788. doi:10.1172/JCI200320514

Westerterp, K. R. (2004). Diet induced thermogenesis. *Nutrition & Metabolism*, *1*(1), 5. doi:10.1186/1743-7075-1-5

Weyer, C., Yudkin, J. S., Stehouwer, C. D., Schalkwijk, C. G., Pratley, R. E., & Tataranni, P. A. (2002). Humoral markers of inflammation and endothelial dysfunction in relation to adiposity and in vivo insulin action in Pima Indians. *Atherosclerosis*, *161*(1), 233–242.

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., … Chen, H. (2003a). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation*, *112*(12), 1821–1830. doi:10.1172/JCI200319451

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., … Chen, H. (2003b). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation*, *112*(12), 1821–1830. doi:10.1172/JCI200319451

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., … Chen, H. (2003c). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation*, *112*(12), 1821–1830. doi:10.1172/JCI200319451

Zechner, R., Kienesberger, P. C., Haemmerle, G., Zimmermann, R., & Lass, A. (2009a). Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *Journal of Lipid Research*, *50*(1), 3–21. doi:10.1194/jlr.R800031-JLR200

Zechner, R., Kienesberger, P. C., Haemmerle, G., Zimmermann, R., & Lass, A. (2009b). Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *Journal of Lipid Research*, *50*(1), 3–21. doi:10.1194/jlr.R800031-JLR200

Zechner, R., Kienesberger, P. C., Haemmerle, G., Zimmermann, R., & Lass, A. (2009c). Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *Journal of Lipid Research*, *50*(1), 3–21. doi:10.1194/jlr.R800031-JLR200

Zechner, R., Kienesberger, P. C., Haemmerle, G., Zimmermann, R., & Lass, A. (2009d). Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *Journal of Lipid Research*, *50*(1), 3–21. doi:10.1194/jlr.R800031-JLR200

Zechner, R., Kienesberger, P. C., Haemmerle, G., Zimmermann, R., & Lass, A. (2009e). Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *Journal of lipid research*, *50*(1), 3–21. doi:10.1194/jlr.R800031-JLR200

Zechner, R., Kienesberger, P. C., Haemmerle, G., Zimmermann, R., & Lass, A. (2009f). Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *Journal of Lipid Research*, *50*(1), 3–21. doi:10.1194/jlr.R800031-JLR200

Zechner, R., Kienesberger, P. C., Haemmerle, G., Zimmermann, R., & Lass, A. (2009g). Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *Journal of Lipid Research*, *50*(1), 3–21. doi:10.1194/jlr.R800031-JLR200

Zhan, T., Poppelreuther, M., Ehehalt, R., & Füllekrug, J. (2012). Overexpressed FATP1, ACSVL4/FATP4 and ACSL1 Increase the Cellular Fatty Acid Uptake of 3T3-L1 Adipocytes but Are Localized on Intracellular Membranes. *PLoS ONE*, *7*(9), e45087. doi:10.1371/journal.pone.0045087

Zimmermann, R., Strauss, J. G., Haemmerle, G., Schoiswohl, G., Birner-Gruenberger, R., Riederer, M., … Hermetter, A. (2004). Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science*, *306*(5700), 1383–1386.