The Effect of Leucine Supplementation on Biomarkers of Mitochondrial Biogenesis and Protein Synthesis in Soleus Muscle of Rats Fed a High-Fat Diet

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The Effect of Leucine Supplementation on Biomarkers of Mitochondrial Biogenesis and Protein Synthesis in Soleus Muscle of Rats Fed a High-Fat Diet
The Effect of Leucine Supplementation on Biomarkers of Mitochondrial Biogenesis and Protein Synthesis in Soleus Muscle of Rats Fed a High-Fat Diet

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Kinesiology

by

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ABSTRACT

Mitochondrial dysfunction is a contributing factor to the advancement of various diseases including obesity, diabetes, and cardiovascular disease. Impairment in mitochondria brings about a diminished mitochondrial number and oxidative capacity. Leucine is known to stimulate muscle protein synthesis through transcription and translation of nuclear DNA leading to increases in mitochondrial biogenesis and fatty acid oxidation. However, the effects of leucine on anabolic factors of the mitochondrial genome, during catabolic conditions are not known.

<bold> PURPOSE </bold>: To determine if leucine supplementation alters the negative effects of a high fat (60%) diet (HFD) on the mitochondrial genome by measuring the gene expression of mitochondrial protein synthesis markers TFAM, mtIF2, and TUFM, and mitochondrial biogenesis regulatory factors PGC-1α and COX-IV.

<bold> METHODS </bold>: Male, Sprague-Dawley rats (n = 30 / dietary treatment) were fed either a control diet (C), control + leucine (CL), high-fat (HF), or high-fat + leucine (HFL) for 42 days. At the conclusion of 42 days, the soleus was extracted and used for analysis. Quantitative PCR was conducted to determine gene expression for PGC-1α, TFAM, mtIF2, TUFM, and COX-IV.

<bold> RESULTS </bold>: mtIF2 gene expression increased 4-fold (p < 0.05) and TFAM gene expression increased 5-fold, with a control diet supplemented with leucine, respectively. mtIF2 gene expression increased 4-fold (p < 0.05) in response to a HFD compared to normal chow. However, TUFM gene expression declined (p < 0.05) in response to a HFD with or without leucine.

<bold> CONCLUSION </bold>: Leucine supplementation did not stimulate an increase in TFAM, mtIF2, and TUFM when exposed to a HFD, but does however stimulate an increase of mitochondrial protein synthesis markers in normal conditions. Indicating enhanced mitochondrial protein synthesis with leucine supplementation, at least under normal conditions.
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CHAPTER 1

INTRODUCTION

Obesity, the most common disorder in the United States and developing countries, is largely due to excess food intake and a lack of physical exercise (9). Major risk factors and comorbidities are associated with obesity including, diabetes, cardiovascular disease, musculoskeletal disorders, and carcinogenesis. With this increase in complications resulting from becoming obese, the individuals affected by this disease are experiencing a loss in high quality and productive life years. These illness states are regularly associated with an abnormal metabolism along with, lessened glucose uptake, modified protein turnover, and dysregulated lipid metabolism (21).

Increasing evidence suggests mitochondrial dysfunction to be a causative factor in many metabolic diseases including, obesity, diabetes, and cardiovascular disease (26, 35, 49, 52). These disruptions are often triggered by an increase flux of fatty acids (FAs), concomitant with the inability to switch carbohydrate fuel supplies, resulting from a high-fat diet (HFD). However, the underlying mechanisms of metabolic adaptation in response to HFD are only beginning to be unraveled, and the cause-and-effect relationship between HFD and mitochondrial dysfunction remain unclear.

Mitochondria play a key role in whole body metabolic homeostasis by regulating several metabolic processes including, oxidative phosphorylation (OXPHOS), FA β-oxidation, reactive oxygen species (ROS), amino acid metabolism, and calcium homeostasis (37). Thus a change in metabolism, regarded as “mitochondrial dysfunction” has not been an uncommon result seen in the skeletal muscle of obese rodents (7, 53, 67) and humans (34, 62). One of the primary means
of increasing skeletal muscle oxidative capacity is through mitochondrial biogenesis (11). Mitochondrial biogenesis is the main process by which an increase in mitochondrial content and improvement of the functional capabilities of pre-existing mitochondria can be made (55).

Interventions aimed to enhance mitochondrial biogenesis, and thereby mitochondrial functions have been reported to modulate insulin signaling and metabolic defects (59). Many strategies for preventing obesity have concentrated on dietary and lifestyle changes, for example, caloric restriction and becoming more active. These studies have revealed that despite the fact that weight loss can be achieved by modified dietary approaches, dieters find it difficult to maintain their weight loss over a certain time period (33). A growing body of data has demonstrated a unique signaling role of the branched-chain amino acid (BCAA), leucine, on improving lipid and glucose metabolism (68). In these cells, leucine increased muscle protein synthesis via activation of the mammalian target of rapamycin signaling pathway (mTOR) (13), stimulated mitochondrial biogenesis (13), as well as fatty acid oxidation (45).

Further data suggests that leucine stimulates SIRT-1, an activator of PGC-1α, which is associated with the initiation of mitochondrial biogenesis and fat oxidation in skeletal muscle cells (17). PGC-1α activates mitochondrial biogenesis and increases OXPHOS gene expression (21) by increasing the transcription, translation, and activation of transcription factors necessary for mitochondrial biogenesis. Among them, transcription factor of activated mitochondria (TFAM) is one of the regulatory factors needed for proper transcription of mitochondrial DNA (mtDNA) (49). In a 2007 study by Turner et al. (69), mitochondrial biogenesis and FA oxidative capacity was shown to be enhanced in skeletal muscle of mice on a HFD.
However, mitochondria are equipped with their own protein synthesis machinery, encompassing 13 protein-encoding genes that are all essential in the function of the electron transport system, and paramount to metabolic health. Two nuclear-encoded proteins, mitochondrial translation initiation factor 2 (mtIF2) and mitochondrial translation elongation factor-Tu (TUFM) have been identified as essential components of the mitochondrial translation process. mtIF2 and TUFM are translated in the cytosol by the canonical translation machinery, and imported to the mitochondria where they are primarily responsible for the translation initiation and elongation processes (47).

Leucine supplementation has been well documented in stimulating certain protein synthesis related anabolic processes. However, the effect of leucine supplementation on the mitochondrion transcriptional and translational machinery in a catabolic situation, caused by HFD has yet to be established. The present study evaluates the impact of a HFD on markers of mitochondrial protein synthesis and mitochondrial biogenesis in rat soleus muscle. We further determined the effect of leucine supplementation on the anabolic factors involved in mitochondrial protein synthesis and mitochondrial biogenesis in the soleus muscle of rats submitted to a HFD.

CHAPTER 2

REVIEW OF LITERATURE

This literature review is divided by topic into 5 main sections: 1) Obesity and the adverse effects on health status, 2) skeletal muscle and the functional changes in response to high-fat feeding, 3) mitochondrial regulation and its role in energy metabolism, 4) mitochondrial biogenesis as it relates to the regeneration of damaged mitochondria, and 5) the anabolic role of leucine in muscle protein synthesis and mitochondrial regulation. This review will include
general knowledge on high-fat feeding and how it has been shown to effect muscle protein
synthesis in previous research, as well as how the mitochondrial dysfunction occurs in muscle
under catabolic conditions. The two components of mitochondrial processes that will be
examined in this study include protein synthesis and mitochondrial biogenesis. Previous
literature on markers of mitochondrial biogenesis and oxidative capacity, (PGC-1α and COX-IV)
and the role these play in mitochondrial biogenesis within skeletal muscle will be reviewed.
Markers of mitochondrial protein synthesis including TFAM, mtIF2, and TUFM, will be
reviewed in their role in the transcriptional and translational processes.

I. Obesity

The rising epidemic of obesity is a leading health concern over the globe. The World
Health Organization (WHO) characterizes obesity as a state of unusual fat mass gathered in
adipose tissue, which may cause extreme health consequences (9). Obesity is the most frequently
encountered metabolic disease across the world (9). In the United States, obesity is currently
prevalent in more than two-thirds of the adult population, with rates anticipated to expand later
on without mediation (54). Research has also shown that a key predictor of high blood pressure,
type 2 diabetes, cardiovascular disease, and premature death are associated with obesity (29).

Body mass index (BMI) is an indirect measure of obesity with a correlation to body fat
mass in children, and adults (51). A BMI value greater than or equal to 30 kg/m² is inferred to as
being obese (75). Among adults, a greater proportion of men (41.2%) are overweight contrasted
with women (28.4%), and women are more probable than men to be obese (75). The changes
that have been brought by industrialization have negative impacts on both diet and physical
activity levels which illustrates a piece of the expanding patterns in obesity over the previous
decade. Despite the poor health outcomes that arise due to excessive intake of energy-dense foods, the trend for obesity shows no sign of plateauing. Therefore, it is imperative to understand the physiological changes associated with the onset of obesity and determine an intervention that will prevent or attenuate impaired metabolic function.

Complications and Comorbidities Associated with Obesity

Complications of obesity in adults include dyslipidemia, type 2 diabetes, coronary illness, hypertension, disease, and premature death (74). Alongside the expansion in rate of obesity in adults, is complication of obesity infrequently seen in pediatric populace, for example: type 2 diabetes, hypertension, cardiovascular ailment, and metabolic syndrome is getting more pervasive. Due to the expansion in complexities associated with obesity, those who are influenced by this ailment experience a decline in a high quality life when exposed to this sickness in early phases of life (69). It has likewise been recognized that when people are corpulent in their young lives, they encounter more trouble in losing this weight, so the overweight child usually turns into an overweight or obese adult.

Obesity has also been shown to prompt insulin and leptin resistance, which brings about dysfunctional adipose and skeletal tissue (73). At the point when this happens, metabolic dysregulation occurs with hoisted levels of circulating free fatty acids (FFAs), and increased generation and discharge of pro-inflammatory adipokines. If left untreated, can prompt conditions such as liptoxicity, chronic inflammation, hypertension, atherosclerosis, and cardiovascular disease (68). As BMI increases, systolic and diastolic blood pressure additionally expand (63). An obese individual normally shows a debilitated lipid profile, where triglycerides are raised, HDL-cholesterol concentration is lessened, and low-density lipoprotein levels are
raised (63). It is likewise vital to note that visceral fat amassing is more risky for the advancement of cardiovascular ailment than fat on the extremities. Additionally, there is a link between obesity and advancement of type 2 diabetes with an increased risk of developing pre-diabetic conditions as an aftereffect of impeded glucose tolerance, and insulin resistance in the tissues (69).

The huge number of complications and comorbidities connected with obesity not only cause human suffering and diminished personal satisfaction in a few cases, but this also decides the surprising expenses connected with obesity. The assessed expenses of obesity extend from 6% to 16% of aggregate medicinal services in the United States (54). This expense is likely going to climb with ease of access to unhealthy sustenance’s (54) and the decline of physical activity around occupants of the United States, as well as in developing nations. Due to the rising obesity epidemic and its connected comorbidities, it is critical to expand research addressing the issue.

**Etiology of Obesity**

There are many genes that are connected with energy metabolism, and there is now interest toward variables that have been illustrated to partake in the commitment of an individual’s vulnerability to put on weight and create comorbidities connected with obesity. The recent investigated supporters to the surge in obesity include diseases, lack of sleep, and a diminishment in an assortment of surrounding temperatures, mitochondrial dysfunction, aging, and assortative mating alongside epigenetics (16). This research focus has recently come of interest because of the now known effect of different supplements, and environmental compounds on the mitochondrial genome, which can prompt more serious disease processes.
II. Skeletal Muscle

Structure and Function

Skeletal muscle is a standout amongst the most specialized structures in the whole body framework. There are in excess of 400 skeletal muscles, which constitute 40 percent to half of the total body mass, therefore making it the largest organ in the human body (73). Skeletal muscle cells (myofibers) are the fundamental unit of a singular skeletal muscle, and are comprised of many nuclei. Encompassing an individual nucleus is a district of cytoplasm known as the myonuclear domain (79). The nucleus assumes a critical part in the gene expression for its encompassing myonuclear area.

Muscle fibers can adjust to quick or moderate twitch activity. This variation starts from a functional demand of the myofibers, and is fortified by incitement of fiber type-specific contractions (79). There are two principle contractile fiber types: glycolytic (type I) and oxidative (type II) fibers. Glycolytic muscles, for example, the gastrocnemius, are basically made out of fast twitch fibers and produce energy by means of anaerobic metabolic techniques. Oxidative muscles, such as soleus, are slower to contract yet oppose fatigue at a slower pace by means of oxidative metabolic methods (10).

The peripheral region of the central nervous system (CNS) controls the skeletal muscles. In this manner, skeletal muscle is under cognizant or voluntary control. Voluntary movement is critical to the survival of humans, and is contributed to an endless show of proteins found in the mature skeletal muscle myofiber (79). Skeletal muscle is critical for exercises of day-by-day living, and obliges the facilitated regulation of inflammation, protein synthesis, and protein degradation for ideal muscle development (73). The three primary functions of the skeletal
muscle are force production of body movement and respiration, posture support, and body heat processing (73).

Adenosine triphosphate (ATP) is a molecule that stores energy inferred from nourishments, and exchanges the energy to cells for biochemical processes (5). All muscles require ATP to produce contractions and movement. The capacity of the skeletal muscle cell is energized by glucose and lipid oxidation. Under ordinary augmenting conditions, glycolytic muscles use glucose, while oxidative muscles depend all the more on lipids (64). During physical activity, muscle cells are enacted and enhance their contractile performance. Conversely, muscles that do not undergo regular activity consider the opposing effect.

Protein Synthesis

Protein is an essential macromolecule, which helps make the structural component of cells, and is used within many biologic reactions (2). Proteins are composed of a vast array of smaller units called amino acids, connected in long chains. Twenty diverse amino acids are required in a particular order to characterize every protein’s shape and function. There are a multitude of specialized proteins that are involved in cellular activity including, insulin and muscle cell filaments. All things considered, proteins assume an essential part in the shaping of muscle tissue, and help support a healthy, and dynamic body.

Protein synthesis is an event that occurs in all the cells of the body via transcription and translation processes. Messenger ribonucleic acid (mRNA) is synthesized in the nucleus where it receives the instructions from DNA (transcription) and transfers the code into the cytoplasm where protein synthesis occurs. Ribosomal RNA (rRNA) specializes in the manufacture of ribosomes, the organelle that mRNA binds to in order to transfer the genetic code. Transfer RNA
(tRNA) resides in the cell cytoplasm, and is responsible for supplying the ribosomes with amino acids for protein synthesis (translation). After completion, the protein is removed from the ribosome and used as a structural component of a cell, or released to perform its proper function. A decline in skeletal muscle protein synthesis may have a negative impact on biological processes, and thus affect optimal health.

III. Mitochondrial Regulation

Mitochondrial Dysfunction in Health and Disease

Within each cell are tiny sub-cellular organelles known as, mitochondria, that extract substantial amounts of energy from consumed nutrients, in order to fuel each individual cell and ultimately the whole human body (5). Mitochondria assume a crucial role in cellular metabolism, energy homeostasis, cell signaling, and apoptosis (25). Without mitochondria, the production of energy and all cellular functions would be compromised. Hence, maintaining abundant, functional mitochondria is essential to sustain life.

An expansion of literature distinguishes mitochondrial dysfunction as a contributing factor to various pathophysiologies (25, 78). Given its significant role in the cell, it comes as no surprise that harm to mitochondria ultimately prompts disease (15). Case in point, mitochondrial dysfunction is perceived as a causative element in a wide assortment of disease states, which include obesity, diabetes, cardiovascular disease, cancer, and dementia (15, 78). The commonality amongst these disease pathophysiologies comes from the generation of ROS that bring about the accumulation of damage to mitochondrial DNA (mtDNA) (25). This eventually prompts mitochondrial dysfunction, resulting in more ROS; accordingly sustaining an endless loop (25). This mixture of oxidative stress and mitochondrial dysfunction is common to various
conditions; thus, the identification of factors that can attenuate oxidative stress and mitochondrial function is significant (22).

**Mitochondrial Structure and Function**

Mitochondria have a double membrane, bringing about different compartments inside the organelle. The semi-porous external mitochondrial membrane encases the organelle, differentiating it from the cytosol (27). The intermembrane space is located between the outer and inner membrane, and serves as an area for protein transport. Imbedded in the mitochondrial inner membrane are the enzymes responsible for extracting energy from food via ATP synthases and oxidative phosphorylation (OXPHOS) (32). The inner membrane is further compartmentalized to structure cristae, which builds the surface region in this way expanding the organelles capacity to synthesize ATP (32). Lastly, the mitochondrial matrix is the space within the mitochondrial membrane, and is the site of the citric acid cycle (also known as Krebs cycle) and also unsaturated fat oxidation (32).

Fuel substrates such as, glucose, lipids, and fatty acids combine with oxygen to produce energy via OXPHOS and electron transport system (ETC), which is used to synthesize ATP. The ATP then spreads through the cell, and the required energy stored within the phosphate bonds of ATP is released to support cellular functions (15). The mitochondrial ETC consists of a series of redox reactions, where the substrates NADH and FADH₂ donate electrons to a terminal electron acceptor, oxygen. The outcome of electron transport is to build up a proton gradient outside the inner mitochondrial membrane. The resulting proton gradient navigates the inner mitochondrial membrane back to the matrix, thus activating the membrane ATP synthase and producing 80% of the cellular ATP.
OXPHOS involves an electrochemical gradient, whereby high-energy electrons of NADH are converted into ATP’s high-energy bonds. OXPHOS pathways do not require oxygen to produce ATP. The OXPHOS system consists of 5 multi-protein enzyme complexes (I-V) that all contribute in transporting electrons and proteins. The three protein pumps include complexes one, three, and four (42). Cytochrome c oxidase or complex IV (COX-IV) is an enzyme that catalyzes the final step in the electron transfer, and is considered a major regulation site for OXPHOS (42).

While the primary responsibility of mitochondria is to produce ATP, this organelle is vital for various cell processes including cellular energy metabolism, cell signaling and apoptosis (25). As the primary site of citric acid cycle, lipolysis, and OXPHOS, mitochondria contain enzymes vital for the synthesis of lipids, cholesterol, heme, and assume a basic part in amino acid metabolism (25). Also, ROS are a product of mitochondria during OXPHOS. This controlled era of ROS by the mitochondria is vital for both cell signaling and apoptosis (25).

**Mitochondrial Protein Synthesis**

Each mitochondrion in a cell comprises its own genome (mtDNA), which is entirely separate from the DNA found in the nucleus (nuclear DNA). The mtDNA plays a key role in the expression of the respiratory chain. The components that make up the mitochondrial genome include, a large rRNA, small rRNA, proteins, and 22 tRNAs that are necessary for the translation of the RNA made by the mitochondria (32). Muscle mitochondrial content is a key factor of aerobic exercise capacity in healthy subjects.

The mitochondrial genome codes for a very limited number of proteins, and rely on the nuclear DNA to provide the structural and enzymatic machinery required for protein synthesis.
There are more than 95% of proteins encoded in the nuclear DNA, synthesized in the cytoplasm, and transported to the mitochondria (21). Despite the limited amount of protein in the mtDNA, each protein is paramount in the expression of the respiratory chain, and thus mitochondrial health. The mitochondrial genome undergoes the same transcription and translation processes as nuclear DNA, but consists of different control proteins that are significant in the proper function of protein synthesis. Enzyme and transcription factors encoded in the nucleus direct the transcription of mtDNA. Transcription factors are regulatory proteins responsible for the expression of nuclear genes encoding respiratory chain proteins (21).

The initiation of transcription begins at the heavy (P_H) and light (P_L) strand promoter sites of the DNA strand. The P_H strand is responsible for coding most of the 13 genes that are encoded by the mtDNA, as well as, tRNA’s that are needed for proper translation into proteins (73). Transcription factors such as, mitochondrial transcription factor A (TFAM) are needed to ensure proper transcription of mtDNA, and of genes involved in the respiratory complexes (73). Both, P_H and P_L promoter sites, share an upstream enhancer that denotes the recognition site for TFAM. Transcription is then stimulated through specific binding to these recognition sites.

TFAM also plays a role in stabilizing and maintaining the mitochondrial genome through nonspecific binding to random sites on mtDNA at a high affinity. Previous findings on TFAM knockout mouse concluded that, without TFAM embryonic cells were destructible, and there was a depletion of mtDNA content (71). Thereafter, it was suggested that TFAM levels correlate with increased levels of mtDNA in ragged-red muscle fibers (72). Ragged-red muscle fibers are associated with clumps of “diseased” mitochondria. Another study established a correlation between lower TFAM expression levels with decreased mtDNA levels in cells that contained depleted mtDNA (65).
The mitochondrial translation machinery relies on the nuclear genome that encodes protein necessary for proper translation. The protein components necessary for translation include, tRNA synthetases, mitochondrial ribosomal proteins, and initiation, elongation, and termination translation factors (2). The first qualification that must be met in order for translation to take place is, the mtDNA must be present, maintained, and transcribed (2). Secondly, proteins encoded in the nuclear genome that are responsible for proper mitochondria function have to be imported from the cell cytoplasm (2).

Translation Factors

A late report by Mercader et al. (47) implicates two key proteins for the mitochondrial translation process as novel applicant genes in human type 2 diabetes. These proteins are known as, mitochondrial translation initiation factor 2 (mtIF2) and mitochondrial translation elongation factor-Tu (TUFM). mtIF2 and TUFM display essential obligation regarding the translation initiation and elongation processes (21). mtIF2 and TUFM are exceedingly monitored nuclear-encoded proteins interpreted inside the cytosol by the standard translation machinery and imported to the mitochondria (21). There, they are fundamental components of the mitochondrial translation process inside the mitochondrial matrix (21). Right now, it is accepted that the start of interpretation happens upon mtIF2 binding to the mitoribosomal small subunit (SSU) which transports fMET-tRNA to the SSU at the aminoacyl acceptor site for interpretation (translation) of the mRNA, taking after edit TUFM is then comparably discharged from the unit through GTP hydrolysis permitting an alternate tRNA-TUFM to come in (21). This methodology proceeds to the end of the mRNA by means of a comparable stop codon mechanism to nuclear translation. Smits et al. (66) have beforehand distributed a more detailed audit of this procedure. The exact regulation of each one stage and protein included is still undetermined. In any case, it creates the
impression that various translation variables display potential binding sites to the mtIF2 and TUFM promoters, moreover Hornbeck et al. (28) noted 27 anticipated binding sites for posttranslational modification of TUFM. It thusly makes sense that complete mitochondrial biogenesis requires the upregulation of the mitochondrial translation machinery so as to include these key components.

IV. Mitochondrial Biogenesis

Mitochondrial functions are controlled, to a limited extent, by progressions in mitochondrial mass, such that diminished mitochondrial number contributes to a loss of function (68). Strict control over mitochondrial progress also allows this organelle to have changing reactions based upon adjustments in the environment such as, physical activity, availability of nutrients, and low temperature (68). This control is accomplished through mitochondrial biogenesis, which is a fundamental living methodology needed for development and advancement, in addition to meet the variable energy necessities of the cell (25). The synthesis, import, and integration of macromolecules to the mitochondrial reticulum is required in order for mitochondrial biogenesis to occur. Mitochondrial biogenesis is further convoluted by the presence of double genomes, and the fundamental replication of the mitochondrial genome (25).

Mitochondria are produced from the transcription and translation of encoded genes in both the nuclear genome and mitochondrial genome. Correlation of mitochondria in numerous mouse tissues uncovered noteworthy congruity between protein levels and gene expression levels as measured by mRNA. This recommends that the tight control of mitochondrial biogenesis is to a great extent attained through transcriptional regulation (25). A gene is a specific region in the DNA, containing a message to produce various chemical substances. The
Mitochondrial genome is a circular segment of DNA that comprises of 37 known genes (21, 25). Of those 37 genes encoded by mtDNA, 13 encode for enzymes involved in OXPHOS, 22 encode tRNA, and two encode mitochondrial rRNA (21).

Peroxisome proliferator activated receptor gamma co-activator alpha (PGC-1α) is often referred to as the “master regulator” of mitochondrial biogenesis (21), and is known to be the driving factor in forming oxidative type I fibers, and interrelate with many transcription factors (76). Increased expression of PGC-1α in myotubes strongly induces mitochondrial biogenesis by activating downstream biomarkers such as, nuclear respiratory factor 1 (NRF-1) and (NRF-2), which initiate expression of TFAM (76). The key role of PGC-1α in mitochondrial biogenesis has been obviously exhibited in transgenic studies (10, 41, 43). The overexpression of PGC-1α in skeletal muscle brings about increased mitochondrial abundance, gene expression, and enhanced physical performance (43). Interestingly, PGC-1α null mice showcase diminishes in mitochondrial gene expression and debilitated mitochondrial function (4, 41). Further evidence has shown PGC-1α to stimulate fatty acid oxidation, as well as improve insulin sensitivity in rats in a stressful environment (5).

Impaired mitochondria and decreased mitochondrial density, and mtDNA content in high-fat diet-induced obesity are closely linked to the down regulation of PGC-1α and its downstream targets (60). Another study conducted on male rats reported that high-fat diet feeding induced skeletal muscle oxidative capacity by increasing PGC-1α, and the number of mitochondria in the cell (69). Moreover in the soleus muscle of high-fat diet-induced obese mice, COX IV levels, PGC-1α, NRF-1, and TFAM gene expression were decreased, as well as, oxygen consumption and ATP production (70). Taken together, these data indicate that PGC-1α directly relates external stimuli to the regulation of mitochondrial biogenesis and function.
While PGC-1α manages the expression of various mitochondrial genes, its own expression is directed by the energy needs of the cell (22). For instance, PGC-1α is communicated in a tissue-specific way, showing higher expression levels in tissues with higher energy requirements (22). Also, PGC-1α expression and activity is delicate to outer indicators of increased energy needs (22). For example, Greene et al. (21) observed an increased in PGC-1α expression following physical activity. Moreover, the action of PGC-1α is weakened by posttranslational adjustments of AMP-activated protein kinase (AMPK) and sirtuin1 (SIRT1), which are themselves, enacted by an expansion in cell energy needs (37, 56). This by and large infers that PGC-1α is a key impact in the long haul adjustment to the changing energy needs of the cell (25).

**Mitochondrial Biogenesis Assessment**

Mitochondria are exceptionally polymorphic, displaying structural variants that are dependent upon cell type, metabolic needs of the cell, and the cell cycle stage (25). Correspondingly, mitochondrial mass shifts around different organisms and tissue types, and is particularly needy upon the vitality necessities of the cell (25). One of the essential means by which mitochondrial capacity may be upgraded is through the biogenesis of new mitochondrial components (21). Miller et al. (48) suggested that the utilization of mitochondrial protein synthesis rates (mtFSR) may be the closest genuine measure of mitochondrial biogenesis, as these measures portray novel addition of protein components to the mitochondria in “real time". Given that control of mitochondrial biogenesis is accomplished through transcriptional regulation (25, 50), gene expression levels of key mitochondrial regulatory and component genes should be utilized to affirm changes in mitochondrial number instead of size (68). For instance, an upregulation in the expression of PGC-1α and/or NRF-1 are symbolic of expanded
mitochondrial biogenesis (68). The branched-chain amino (BCAA), leucine, essentially upregulates mitochondrial biogenesis as measured by gene expression levels in skeletal muscle cells (68).

V. Leucine

The inherent rise in obesity has sparked an interest in treatments and prevention methods that will reverse or attenuate mitochondrial dysfunction. Aligned with these strategies, BCCAs have shown to promote health and enhance physical performance (6). Leucine, isoleucine, and valine are defined as BCAAs, a gathering of essential amino acids that assume critical parts in protein synthesis and energy production (67).

Of the BCAAs, leucine is the most broadly explored because of its differing physiological roles in mitochondrial function, protein synthesis, exercise recovery, glucose homeostasis, and insulin activity. In humans, BCAAs comprise between fifteen and twenty-five percent of total protein intake, and embody forty percent of the essential amino acids in body protein (2, 6). As essential amino acid, the BCAAs must be acquired through eating, and dairy items are one of the wealthiest wellsprings of BCAAs and of leucine specifically.

Leucine and Protein Synthesis

Both in vivo and in vitro studies have demonstrated leucine to have a unique signaling role in the cellular energy metabolism of normal skeletal muscle cells. Leucine is an activator of mammalian target of rapamycin (mTOR), a protein responsible for inducing muscle protein synthesis (5). Muscle protein synthesis is stimulated by leucine through mTOR-dependent and-independent mechanisms (12). Leucine is detected through mTOR, and plays a role in the
initiation of translation during protein synthesis. Leucine acts through mTOR independently by activating eIF4G (eukaryotic initiation factor-4G) thereby mediating protein synthesis in the muscle (13, 17). Under a dependent-mTOR mechanism, leucine associates eukaryotic initiation factor-4E (eIF4E) with S6 ribosomal protein to stimulate protein synthesis (17). Dietary supplementation with leucine has been shown to stimulate protein synthesis via insulin-independent mechanisms.

Leucine has also been recognized as a key substrate in fatty acid oxidation and mitochondrial biogenesis in murine myotubes (67, 70). A previous study proposed leucine to regulate adipocyte lipid metabolism, and possibly serve to induce an increased flux of lipids to skeletal muscle, thereby providing the energy fuel to support leucine-stimulated protein synthesis (68). This “energy partitioning” effect was also seen in Zemel et al. (77), with leucine supporting fatty acid oxidation in adipocytes concomitant with activating mTOR and thus, increasing muscle protein synthesis. Furthermore, a 30% increase in mitochondrial mass in adipocytes, and 50% in skeletal muscle cells was observed following leucine stimulation (77).

Leucine and Mitochondrial Biogenesis

Preceding information displays a mechanism, by which leucine is assisted, by its metabolite, β-hydroxy-β-methylbutyrate (HMB) to stimulate mitochondrial biogenesis independent of mTOR (2). The process begins with the metabolism of leucine into α-ketoisocaproic acid (KIC) via an aminotransfer chemical reaction. Recent data demonstrate that leucine is dependent upon SIRT1, an activator of PGC-1α, to initiate mitochondrial biogenesis within these cells. Furthermore, leucine was shown to increase the gene expression of SIRT1, and mitochondrial component genes, including PGC-1α and NRF-1 (8). These genes provide
further verification that leucine signaling lead to an increase in mitochondrial number, rather than mass.

The benefits of BCAA supplementation, leucine specifically, is known to promote mitochondrial wellbeing and possibly diminish oxidative stress (6). Supplementary data revealed, long-term administration of BCAA rich eating regimen brought about a noteworthy expand in the lifespan of male mice, supplemented by a simultaneous increase in mitochondrial biogenesis and lessened oxidative damage in skeletal and heart muscle (6).

Given the evidence on the seemingly diverse effects of leucine, the interest in dietary leucine supplementation still remains prominent in research studies today. Such excitement is due to leucine appearing as a potent activator for protein synthesis, mitochondrial biogenesis, and lipid oxidation, which is a significant feature in understanding how skeletal muscle grows, and regulation of metabolic health. However, the extent of leucine supplementation on anabolic factors of the mitochondrial genome in skeletal muscle during HF feeding, to our knowledge has yet to be fully elucidated. Considering the involvement of leucine in modulating mitochondria abundance and function, determining the role of leucine in an obesogenic environment can serve as a benefit for improving metabolic health, physical performance, and disease status.

CHAPTER 3
SPECIFIC AIMS

Diet-induced obesity is caused by excessive intake of dietary fat over a period of time, and is associated with the development of type 2 diabetes and metabolic dysfunction (9). Mitochondria, the powerhouse of the cell, carries out cellular respiration by combining oxygen and fuel substrates such as, fats, carbohydrates, and proteins to be broken down into cellular energy in the form of adenosine triphosphate (ATP). The addition of new mitochondrial units,
and proteins is important to the ability to enhance total cell mitochondrial function. In obesity, there are a decreased number of mitochondria and oxidative capacity in skeletal muscle, thereby leading to dysfunction muscle metabolism.

The transcriptional co-activator peroxisome proliferator-activated receptor γ co-activator 1 alpha (PGC-1 α) is commonly expressed in high oxidative tissues such as, the soleus muscle, and is known as the ‘master regulator’ of mitochondrial biogenesis (21). PGC-1α is responsible for binding to transcriptional factors that stimulate the activation of gene transcription and translation involved in mitochondrial biogenesis (21). Overexpressed levels of PGC-1α may negatively affect the activation of the downstream transcription genes, thus causing impaired mitochondrial protein synthesis in skeletal muscle.

Anabolic factors that regulate mitochondrial biogenesis and fatty acid oxidation in muscle include, COX IV, TFAM, mtIF2, and TUFM. A study on lean and obese, insulin-resistant Zucker rats showed an increase expression of mtIF2 following exercise, however obese animals were resistant to changes in TUFM, suggesting impaired elongation (21). Along these lines there is now preparatory confirmation showing atypical regulation of exercise-induced gene expression of mtIF2 and TUFM in diet-induced insulin resistance in mice.

Leucine is an essential amino acid that has been shown to attenuate diet-induced obesity (37, 60), promote fatty acid oxidation (12), and improve mitochondrial biogenesis in skeletal muscle (61). Supplementation with leucine has been recognized as a key mediator during chronic inflammation by regulating glucose homeostasis and improving muscle protein synthesis (17). Prominent research has been done on leucine supplementation in HFD feeding; however, it is unknown how leucine affects biomarkers of the mitochondrial genome in response to a HFD.
Therefore, the central hypothesis of this proposal is: leucine supplementation induces an increased expression of mitochondrial biomarkers, thereby improving mitochondrial biogenesis and mitochondrial protein synthesis in skeletal muscle of HFD-fed rats.

The purpose of this study was to determine the effect of leucine in HFD’s by targeting the biomarkers responsible for mitochondrial biogenesis and protein synthesis in the soleus muscle.

**Aim 1. Determine the effect of leucine supplementation on markers of mitochondrial biogenesis in rats fed a high-fat diet.** We hypothesize that a high fat diet supplemented with leucine will induce mitochondrial content and density via elevated PGC1-α and COX IV levels. Male, Sprague-Dawley rats were fed either a HFD (60% fat) or a control diet for 42 days in order to generate diet-induced obesity. Animals were randomized into treatment groups (n = 30/group): 1) control/no leucine; 2) control/leucine; 3) high fat/ no leucine; 4) high fat/leucine. Body weight and food intake were measured daily. At the conclusion of 42 days, animals were sacrificed and the soleus tissue was isolated to measure the gene expression of biomarkers of mitochondrial biogenesis. RNA isolation and real time PCR were utilized to measure gene expression of PGC1-α and COX IV in the soleus tissue.

**Aim 2. Determine the effect of leucine supplementation on biomarkers involved in mitochondrial protein synthesis in rats fed a high fat diet.** We hypothesize that a high fat diet supplemented with leucine will induce transcription and translation activity via elevated TFAM, mtIF2, and TUFM levels. Experiments will examine the transcription factors that are co-activated by PGC1-α including TFAM, mtIF2, and TUFM. This will allow investigators to determine the effect of HFD’s on muscle protein synthesis and where this effect occurs. We will
use RNA isolation and real time PCR to measure gene expression of TFAM, mtIF2, and TUFM in the soleus tissue.

CHAPTER 4

METHODS

Animals and diets

Eight-week-old male Sprague-Dawley rats (Harlan-Teklad), weighing 220 ± 2.0g at the beginning of the experiment, were singly housed in a secure, temperature and humidity-controlled environment. Animals were kept on a 12:12 h reversed light-dark cycle (light 1900 – 0700), and were fed commercial pelleted diet and water ad libitum for 9 days. Once the experiment began, rats were randomly assigned into treatment groups (n = 4-5/group): 1) control + glycine; 2) control + leucine; 3) high fat + glycine; 4) high fat + leucine. Rats were fed macronutrient contents of 60% carbohydrate (CHO), 23.7% protein (PRO), 15.9% fat (control; Table 2) or 16.9% CHO, 23.4% PRO, 60% fat (HF; Table 2) containing 32 g of either glycine or leucine per kg of feed. A customized daily feeding schedule as previously reported Baum et al. (6), was used to mimic human eating behavior. Throughout the experimental period, rats had ad libitum access to water.
Table 1

_Diet composition_

<table>
<thead>
<tr>
<th>Ingredient (g/kg)</th>
<th>Control (+ glycine)</th>
<th>Control (+ leucine)</th>
<th>High-Fat (+ glycine)</th>
<th>High Fat (+ leucine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>265</td>
<td>265</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
<td>4</td>
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<td>Corn Starch</td>
<td>397.5</td>
<td>397.5</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Maltodextrin</td>
<td>100</td>
<td>100</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Soybean Oil</td>
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<td>70</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Lard</td>
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<td>0</td>
<td>310</td>
<td>310</td>
</tr>
<tr>
<td>Cellulose</td>
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<td>Vitamin Mix</td>
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<td>10</td>
<td>21</td>
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</tr>
<tr>
<td>Choline Bitartrate</td>
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<td>Calcium Phosphate, diabasic</td>
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<td>TBHQ</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Glycine</td>
<td>32</td>
<td>0</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Leucine</td>
<td>0</td>
<td>32</td>
<td>0</td>
<td>32</td>
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</table>

Table 2

_Total kCal and percent energy_

<table>
<thead>
<tr>
<th>Macronutrient Component</th>
<th>Control Group</th>
<th>High-Fat Group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Kcal/Kg</td>
<td>% Energy</td>
</tr>
<tr>
<td>Carbohydrate (CHO)</td>
<td>2390</td>
<td>60</td>
</tr>
<tr>
<td>Protein (PRO)</td>
<td>940</td>
<td>23.7</td>
</tr>
<tr>
<td>Fat</td>
<td>630</td>
<td>15.9</td>
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<tr>
<td></td>
<td>872</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>1204</td>
<td>23.4</td>
</tr>
<tr>
<td></td>
<td>3060</td>
<td>60</td>
</tr>
</tbody>
</table>

_Body weights and food intake_

Body mass (BM) and food intake were monitored daily. Before feeding breakfast, rats were placed in a container on a scale and measurements were recorded in grams (g). Rats were given a set portion of either a chow diet (4g) or high-fat (3.1g).
Tissue collection

After 42 days of feeding, rats were sacrificed and tissue was collected. Soleus muscles were removed and immediately frozen in liquid nitrogen. Samples were stored at -80°C for later analysis.

RNA isolation, cDNA synthesis, and quantitative real time PCR

Trizol reagent (Life technologies, Grand Island, NY, USA) was used to extract total RNA as suggested by the manufacturer. The extracted RNA was DNase treated, and the concentration and purity was determined spectrophotometrically using a (A_{260}/A_{280}) ratio of ≥1.6. cDNA was reverse transcribed from 1µg of total RNA using the Superscript Vilo cDNA synthesis kit (Life Technologies, Carlsbad, CA, USA) according to manufacturer instructions. Real Time Polymerase Chain Reaction (PCR) was carried out per manufacturer’s instruction using the StepOne Real-Time PCR System (Life Technologies, Applied Biosystems, Grand Island, NY), and results were analyzed using StepOne Software. Amplification of cDNA was performed in 12.5 µL TaqMan Universal PCR Master Mix (Applied Biosystems), 1µL cDNA, 9µl RNase-free water, 1.25µL 18S primer (VIC), and 1.25 µL primer (FAM) (for appropriate target gene) in a final volume of 25 µL /well. Samples were incubated at 95°C for 4 min, followed by 40 cycles of denaturation, annealing and extension at 95°C, 55°C, and 72°C respectively. TaqMan fluorescence was measured at the end of the extension step each cycle. Fluorescence labeled probes for PGC-1α, TFAM, TUFM, mtIF2 (FAM dyes), and 18s (VIC dye) were purchased from Applied Biosystems and were quantified with TaqMan Universal mastermix. Fluorescence labeled probes for COX-IV was quantified with Cyber Green Universal mastermix. The ΔC_t value [C_t(FAM) - C_t(VIC)] was calculated for each sample, using the 18s C_t as the endogenous
control. Final quantification of gene expression was calculated using the ΔΔCt method: 
\[ C_t = [\Delta C_t \text{(calibrator)} \quad – \quad \Delta C_t \text{(sample)}]. \]
Relative quantification was calculated as \(2^{-\Delta\Delta Ct}\).

**Statistical analysis**

The independent factors in this study were diet (C vs. HF), and treatment (leucine vs. no leucine). Dependent variables of interest included mRNA content of PGC-1α, TFAM, mtIF2, TUFM, and mitochondrial target protein COX-IV. A diet by treatment (2x2) ANOVA was used to analyze data regarding each dependent variable of interest. Statistical significance was set with an alpha value of \(p < 0.05\). When significant F ratios were found, a two-tailed Student’s \(t\) test post hoc analysis was used to distinguish among means. All data were analyzed using commercial software (SAS, version 9.3, Cary, NC) and all results are reported as mean ± SEM.

**CHAPTER 5**

**RESULTS**

**Muscle Mass and Body Mass Data**

Immediately following extraction the soleus was weighed and recorded. There was a decrease in the wet weight in both the normal and high-fat (HF) groups that were supplemented with leucine compared to their respective controls (Table 3). No significant difference was seen in the ratio of muscle mass to body weight with leucine supplementation in either group. Beginning approximately day 20, HF rats weighed significantly more than the normal rats (\(p<0.05\)) (Figure 1). The rats on a HFD gained significantly more weight (\(p < .0001\)) than those fed a normal diet with values of \((409.07 \pm 3.01 \text{ vs. } 434.52 \pm 3.01)\), respectively (Figure 1).
Figure 1. Body weights of rats fed normal diet (N) or HFD, supplemented with/without leucine for 42 days. Body weights were monitored daily. Values are means ± SEM, n=30. *p < 0.05.

Table 3

Muscle Mass, Body Mass, and Muscle to Body Mass Ratio

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th></th>
<th>High Fat</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Leucine</td>
<td>Leucine</td>
<td>No Leucine</td>
<td>Leucine</td>
</tr>
<tr>
<td>Soleus (mg)</td>
<td>166.3 ± 2.4</td>
<td>161.2 ± 3.1</td>
<td>180.2 ± 2.9</td>
<td>173.8 ± 2.8</td>
</tr>
<tr>
<td>Body Mass (g)</td>
<td>408.1 ± 3.5</td>
<td>410.1 ± 3.9</td>
<td>433.9 ± 4.7</td>
<td>435.2 ± 4.8</td>
</tr>
<tr>
<td>Muscle Mass/Body Mass (mg/g)</td>
<td>0.41 ± 0.01</td>
<td>0.39 ± 0.006</td>
<td>0.42 ± 0.004</td>
<td>0.40 ± 0.006</td>
</tr>
</tbody>
</table>

Note. Data are means ± SEM. The HFD contained 60% fat.

Gene Expression of Mitochondrial Transcription and Translation Factors

A significant increase (p<0.05) in mtIF2 expression was observed in the CL group, with a 4.0 fold increase compared to the control within the same group. Conversely, a blunted response in mtIF2 expression was observed in the HFL rats (Figure 2). Although a mean decrease was
seen in HFL, an interaction was demonstrated in the diets, with a significant increase ($p = 0.018$) in mtIF2 expression in the HF group compared to the normal group.

![Figure 2. Fold change comparison of mtIF2 expression in both normal and HF groups with or without leucine supplementation. *Shows significance within group, + Shows significance between groups.](image)

Similarly, a 5.5 fold increase in TFAM expression was seen in rats on normal and leucine compared to their respective control (Figure 3). Although, without leucine supplementation, TFAM expression was approximately 3.5 fold greater in the HFD rats compared to the N rats.

![Figure 3. Fold change comparison of TFAM expression in both normal and HF groups with or without leucine supplementation. A main effect ($p= 0.01$) in the diet was seen in TUFM gene expression between the normal and HF groups. Within the normal group, regardless of the treatment, TUFM gene](image)
expression was up regulated. However, a 1.0 fold decline in TUFM expression was demonstrated in the HF group.

![Graph showing TUFM expression comparison](image)

**Figure 4.** Fold change comparison of TUFM expression in both normal and HF groups with or without leucine supplementation

+ Shows significance between groups

**Effects of Leucine Supplementation on Mitochondrial Biogenesis Markers in Skeletal Muscle**

Markers involved in mitochondrial biogenesis including, PGC-1α and COX IV, were measured as to allow for quantification of the response to a HF diet. It was observed that in the CL group, PGC-1α gene expression was up regulated, however a trend decline (p=0.0724) was demonstrated in the HFL group (Figure 5). The HF group had a slight increase (0.5 fold increase) in PGC-1α expression compared to the normal group.

COX-IV, a marker for mitochondrial volume, was unaffected by leucine supplementation in the HFD group (Figure 6). Although, no significance was found, COX-IV mRNA levels
increased 4.0-fold in the normal group supplemented with leucine compared to their respective controls.

Figure 5. Fold change comparison of PGC-1α expression in both normal and HF groups with or without leucine supplementation.

Figure 6. Fold change comparison of COX IV expression in both normal and HF groups with or without leucine supplementation.
*Shows significance within group
CHAPTER 6
DISCUSSION

It is presently perceived that nutrients can interrelate with transcription factors and contribute with biological processes. People respectably fluctuate in their individual reactions to diet regimens, and such approaches can help to look at the impact of nutrition on skeletal muscle function. The mitochondrion has long been involved in the pathogenesis of obesity and metabolic dysfunction (33, 36). Beforehand, it has been demonstrated that a mitochondrial insufficiency exists in obese people (30) and rodents (57). Nonetheless, the results of late studies recommend that there is a separation between mitochondrial dysfunction and the progression of obesity (3). Such a disconnection has led this study to focus on specific anabolic factors associated with the mitochondrial genome, which may contribute to the lack of mitochondrial efficiency.

Under normal conditions, leucine seems to stimulate skeletal muscle mitochondrial biogenesis, as the increase of PGC-1α mRNA suggest. Leucine has been demonstrated to stimulate SIRT-1 activity, an activator of PGC-1α to start mitochondrial biogenesis and ultimately increase energy consumption within these cells. Previous data exhibit leucine's stimulation of mitochondrial biogenesis is independent of mTOR through leucine's metabolite, β-hydroxy-β-methylbutyrate (HMB) (8). The breakdown of leucine is required to elicit the leucine-induced stimulation on mitochondrial biogenesis. Thus, leucine is metabolized via a transamination reaction to α-ketoisocaproic acid (KIC). The two metabolites of KIC, isovaleryl-CoA and HMB, along with leucine have been shown to significantly stimulate SIRT1 activity, while the branched-chain amino acid control valine did not (8). According to these analyses, an increment in mitochondrial number instead of mass is suggested to occur from leucine signaling.
A different sirtuin located in the mitochondria, SIRT3, was shown to modulate SIRT1-dependent pathways. SIRT3 activation leads to stimulation of mitochondrial biogenesis and metabolism, resulting in enhanced fatty acid oxidation and a decrease in ROS production. Recent evidence on SIRT3 null mice have shown constitutive shortages in ATP production (1), and a propensity to develop the metabolic syndrome when exposed to a HFD (24). Such analyses reveal the importance of SIRT3, and indicate a possibility of leucine to modulate SIRT3 as well.

It is notable, that PGC-1α gene expression appears to be already elevated in HF rats compared to the rats in the normal condition. Increased mRNA levels of PGC-1α, a master regulator of mitochondrial biogenesis (29), would involve the enhancement of skeletal muscle oxidative capacity. Review that fibers with a high oxidative capacity create ATP through oxidative phosphorylation in the mitochondrial inner membrane. It takes after that muscle cells, which hold more mitochondria, will have a higher oxidative capacity, and enhanced fatty acid oxidation. However, a disassociation between PGC-1α, enhancement in oxidative capacity (29), and fatty acid oxidation (27) has been accounted for in obese rodents.

Recent evidence indicates that severely damaged or energetically dead mitochondria are cleared from the cell via autophagic degradation through a pathway known as mitophagy (23). The serine/threonine-protein kinase PINK1, accumulates specifically on the outer membrane of mitochondria (18), which then recruits Parkin and the downstream autophagy machinery that directs the defective organelles to lysosomes for degradation (39). The stresses that ultimately result in mitophagy could explain a potential mechanism of which there is an aggregation of “unhealthy” mitochondria associated with HF intake. Such an accumulation would essentially prompt a rise in the content of mitochondrial components, and subsequently be erroneously recognized as indication of mitochondrial biogenesis (29).
Considering the fact that the HFD rats were able to upregulate primary factors regulating mitochondrial biogenesis (PGC-1α) without a non-significant, leucine-induced enhancement of mitochondrial biogenesis (COX-IV), this study aimed to identify potential anabolic factors associated with the mitochondrial genome that may be unresponsive, or seen as rate-limiting in response to leucine supplementation. These data revealed that TFAM and mtIF2, regulators of mitochondrial transcription and translation initiation, respectively, were upregulated in rats fed a normal chow diet when supplemented with leucine. This discovery is novel in reporting that leucine instigates an increase in the expression of TFAM and mtIF2. Indicating enhanced capacity for transcription and translation of mitochondrial encoded genes in normal, healthy conditions. This data also suggests that leucine supplementation could lead to increased metabolic proteins derived from the mtDNA in mammals. Enhanced mitochondrial protein synthesis would likely prompt increased oxidative phosphorylation enzymes, for example, those utilized as a part of ETC, hence providing significant benefits in metabolic health.

Indeed, collecting evidence demonstrates that HFDs are associated with an upregulation of most anabolic processes (23), and increased expression of key mitochondrial enzymes (14, 20). This study revealed similar results showing that rats fed a HFD induced TFAM and mtIF2 mRNA expression. However, TUFM mRNA, the primary factor in mitochondrial translation elongation was not enhanced in the HF group compared to the control group. It has additionally been noted that mitochondrial translation factors are linked to insulin signaling through the NFκB1/IκBKB pathway (38). More specifically, TUFM cohorts with insulin signaling through the acknowledged translation pathway via ribosomal protein s6 (RPS6), eukaryotic translation initiation factor 4E (eIF4E), and eukaryotic translation elongation factor 2 (eEF2) (38).
It subsequently seems likely that such a disturbance in factors that regulate the canonical translation pathway due to exposure to a HFD, can have unfavorable consequences for TUFM. In addition, eIF4E-binding protein is a downstream effector of mTOR that repress translation by binding to mRNA, and has demonstrated sensitivity to diet-induced obesity (40). These data suggest the comparable mechanisms that oversee the expression of the canonical nuclear genome translation pathway, for example, mTOR may be included in the regulation of TUFM, however such an understanding obliges further examination to explain. Taken together these studies provide novel insight into a possible mechanism for mitochondrial dysfunction in skeletal muscle related to the translational response in a HF environment.

It is notable to mention, HFD’s attenuate the effect of leucine on mitochondrial protein synthesis, which could represent a disruption in the signaling process. The lack of leucine-induced TUFM expression is proposed to be due to an impaired response of translation elongation in a HFD, in this way restricting the improvement of mitochondrial proteins. Taking into account recent studies, TUFM is the essential protein, which assumes a basic part in translation elongation, and along these lines the completion of mitochondrial translation (21). Despite the fact that this study did not specifically assess obese animals or protein content, Greene et al. (21) specified a “bottleneck” point when the obese animal has reached a maximum capacity of inducing mitochondrial translation elongation, and consequently neglect to prompt a measure of mitochondrial protein synthesis. Such a hypothesis is because of an increment in TUFM gene expression, however a diminished reduction in TUFM protein content following resistant exercise in obese, Zucker rats.

Notably, a newly identified mitochondrial protein known as, AU- binding homolog of enoyl-coenzyme A (CoA) hydrates (AUH) demonstrates a bi-functional role in RNA-binding
activity and leucine catabolism (17). Furthermore, AUH is associated with fatty acid degradation, and has been demonstrated to have intrinsic hydratase enzymatic activity. Reduction or overexpression of the AUH protein is linked to defects in mitochondrial translation that prompt changes in mitochondrial morphology, diminish mitochondrial stability, biogenesis, and respiratory function. Regulation of leucine catabolism is paramount to monitor sufficient leucine levels for cytoplasmic protein synthesis and to provide anaplerotic substrates to the citric cycle located in the mitochondrial matrix (17). It therefore appears likely that mutations in the AUH protein inhibits leucine catabolism, and mitochondrial fatty acid oxidation resulting in a decline in mitochondrial protein synthesis during HFD conditions.

In summary, excess energy intake can be directly associated with alterations in muscle mitochondrial activity. The rats fed a HFD showed signs of attempted mitochondrial biogenesis indicative of the enhanced expression of PGC-1α. However, Greene et al. (21) observed no enhancement in mitochondrial protein content, despite an induction in PGC-1α gene expression. Thus leading to the assumption that factors downstream of PGC-1α lead to impaired mitochondrial biogenesis in HF-induced rats. It appears that animals exposed to a HFD are able to integrate signals to initiate an adaptive oxidative response (TFAM, mtIF2), but the failed mitochondrial biogenesis may be due to an inadequate mitochondrial translation elongation (TUFM) response. Such an increase in mRNA expression may represent the mitochondrial components trying to compensate for the deleterious effects of HF intake.

This study demonstrates that the BCAA leucine, has the potential to stimulate mitochondrial protein synthesis in skeletal muscles, at least under normal conditions. However, leucine loses its effect on mitochondrial transcription and translation processes during exposure to a HFD. This suggests that during HF feeding, dietary leucine elicits an incomplete response of
mitochondrial protein synthesis as the progression of HFD-induced obesity increases. This is important as it guides future research into the mechanisms behind this dysfunction. On completion of this study it is need to be noted that protein content may be affected by high fat intake and so further studies on protein analysis is warranted. Also, the assessment of a small group number (n = 4/5) may have affected the outcome of significance, therefore a larger group analysis may be needed to detest greater significance on the anabolic factors.
REFERENCES


MEMORANDUM

TO: Janie Baum

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: February 8, 2012

SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: February 20, 2015

The Institutional Animal Care and Use Committee (IACUC) has APPROVED Protocol #12019-
"DIETARY LEUCINE - ROLE IN METABOLISM AND OBESITY PREVENTION". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] prior to initiating the changes. If the study period is expected to extend beyond 02-20-2015, you must submit a new protocol. By policy the IACUC cannot approve a study for more than 5 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cmc/car

cc: Animal Welfare Veterinarian