The Role of the PGC-1α in Intramuscular Lipid Metabolism

Doctoral dissertation

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"We are not made up, as we had always supposed, of successively enriched packets of our own parts. We are shared, rented, occupied. At the interior of our cells, driving them, providing the oxidative energy that sends us out for the improvement of each shining day, are the mitochondria, and in a strict sense they are not ours."

– Lewis Thomas

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List of Abbreviations

ACh:	Acetylcholine
AET:	Aerobic Exercise Training
Akt:	Protein Kinase B
AMPK:	AMP-activated protein kinase
ATGL:	Adipose Triglyceride Lipase
BAT:	Brown Adipose Tissue
BCA:	Bicinchoninic Acid Assay
BSA:	Bovine Serum Albumin
CCO:	cytochrome c oxidase
CDP:	Cytidine Diphosphate
CEH:	Cholesteryl Ester Hydrolase
CS:	Citrate Synthase
CVD:	Cardiovascular Diseases
CypD:	Cyclophilin D
DAG:	Diacylglycerol
eNAMPT:	Extracellular nicotinamide phosphoribosyltransferase
eNOS:	Endothelial Nitric Oxide Synthase
ER:	Endoplasmic Reticulum
ETC:	Electron Transport Chain
FA:	Fatty Acid
FAO:	Fatty Acid Oxidation
FFA:	Free Fatty Acids
FNDC5:	Fibronectin Type III Domain-Containing Protein5
FOXO3a:	Forkhead Box O3a
GAPDH:	Glyceraldehyde 3-Phosphate Dehydrogenase
GPCRs:	G-protein-coupled receptors
HFD:	High-Fat Diet
HIF:	Hypoxia-Inducible Factor
HPLC:	High Performance Liquid Chromatography

HSL:	Hormone-Sensitive Lipase
IECs:	Intestinal Epithelial Cells
IL-1:	Interleukin-1
IMM:	Inner Mitochondrial Membrane
IMS:	Intermembrane Space
IMTG:	Intramuscular Triglycerol
KO:	Knockout
LCFA:	Long-Chain Fatty Acids
MCFA:	Medium-Chain Fatty Acids
MGL:	Monoacylglycerol Lipase
mRNA:	messenger RNA
mTOR:	mammalian Target of Rapamycin
mTORC1:	mTOR Complex 1
NAMPT:	Nicotinamide Phosphoribosyltransferase
nNOS:	neuronal Nitric Oxide Synthase
NO:	Nitric Oxide
NRF1:	Nuclear Respiratory Factor 1
OMM:	Outer Mitochondrial Membrane
OXPHOS:	Oxidative Phosphorylation
PCYT2:	Phosphoethanolamine Cytidylyltransferase
PE:	Phosphatidylethanolamine
PGC-1α:	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha
PKA:	Protein Kinase A
PKC	calcium-dependent protein kinase C
PPARγ:	Peroxisome Proliferator-Activated Receptor Gamma
PVDF:	Polyvinylidene Difluoride
ROS:	Reactive Oxygen Species
RM:	Repetition Maximum

RT-PCR:	Reverse Transcription Polymerase Chain Reaction
SCFA:	Short-Chain Fatty Acids
SD:	Standard Deviation
SDS-PAGE:	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SDH:	Succinate Dehydrogenase
SDHA:	Succinate Dehydrogenase Complex Flavoprotein SubunitA
SEM:	Standard Error of the Mean
Sirt1:	Sirtuin 1
SIRT3:	Sirtuin 3
SNS:	Sympathetic Nervous System
SOD2:	Superoxide Dismutase 2
TAG:	Triacylglycerol
TBST:	Tris-Buffered Saline-Tween-20
TCA:	Tricarboxylic Acid Cycle
TG:	Triglyceride
UCP3:	Uncoupling Protein 3
VEGF:	Vascular Endothelial Growth Factor
VEGF:	Vascular Endothelial Growth Factor
VO2max:	Maximal Oxygen Consumption
WAT:	White Adipose Tissue

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1. Introduction

The worldwide epidemic of metabolic diseases, such as obesity and type 2 diabetes, highlights the importance of understanding the role of skeletal muscle and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in regulating energy metabolism. Skeletal muscle is an adaptive and important tissue for movement and regulation of metabolism, which acts as the largest endocrine organ that regulates metabolic processes and health. This adaptability is mainly controlled by the PGC-1α gene, which is highly expressed in bioenergetically active tissues such as skeletal muscle (Argilés et al., 2016; Iizuka et al., 2014; Schnyder & Handschin, 2015; Swalsingh et al., 2022). The functions of PGC-1α are regulated by complex networks involving enzymes such as AMPK (adenosine 5'-monophosphate-activated protein kinase) and sirtuin 1 (SIRT1), which act upstream signaling of PGC-1α to activate its downstream pathways (J.-Y. Lin et al., 2020; Radak et al., 2020; Cantó & Auwerx, 2009). These enzymes help skeletal muscle cells respond to energy demands by modulating metabolic processes.

Intramuscular triglyceride (IMTG), lipid droplets stored within skeletal muscle fibers, is an important energy source, especially during endurance exercises (Muscella et al., 2020a). Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) are key lipases that regulate IMTG breakdown via the lipolysis process and utilization. The distribution and accumulation of IMTGs significantly affect muscle function, metabolic health, and the development of metabolic disorders (Bergman et al., 2018).

It has been shown that PGC-1α improves the skeletal muscle's capacity to generate energy from IMTG by increasing the number of mitochondria as well as mitochondrial energy-producing enzymes and affecting lipid breakdown enzymes (Rius-Pérez et al., 2020). For instance, the activation of the SIRT1/PGC-1α/Fibronectin Type III Domain-Containing Protein 5 (FNDC5) axis can enhance lipolysis by stimulating lipolytic enzymes (Xiong et al., 2015).

Although PGC-1α has been known for its role in mitochondrial biogenesis, in this study we aimed to explore its specific role in regulating intramuscular triglyceride metabolism. We used 10-month-old male mice, including both wild-type and transgenic C57BL/6-Tg, to study how the overexpression of PGC-1α in muscle affects IMTG metabolism in response to exercise. This research involved 40 middle-aged mice, comparing both

sedentary and exercise-trained groups over ten weeks of intervention. Specifically, this study was designed to evaluate the distinct and simultaneous effects of PGC-1α and endurance exercise on IMTG metabolism. We evaluated mitochondrial function, mitochondrial health markers, lipid metabolism enzymes, and signaling molecules to understand the mechanisms of mitochondrial change and lipid breakdown in skeletal muscle.

This study aimed to improve our understanding of skeletal muscle responses to exercise and PGC-1α overexpression. These findings have the potential to develop optimal exercise techniques for improving metabolic health, as well as the development of treatments for metabolic diseases including obesity, insulin resistance, and diabetes.

2. Literature review

2.1 Structure of the skeletal muscles and energy metabolism

About 40% of the body's total weight is made up of skeletal muscle, which contains a large proportion of the body's protein, between 50% and 75%, including important contractile proteins (Frontera & Ochala, 2015). The main role of skeletal muscles is to generate force and initiate different movements of body parts, so they are known as the main organs responsible for performing physical activity and have an important role in many locomotor functions (Frontera & Ochala, 2015). Skeletal muscle cells are more sensitive to fatigue than cardiac muscle cells, which rely mainly on aerobic metabolism and are therefore more resistant (Frontera & Ochala, 2015). This tissue is also morphologically different from the other cardiac and soft muscle types due to its specific biochemical features and structure. It has a linear shape because of the distribution of slow-twitch red oxidative and fast-twitch white glycolytic fibers. Slow-twitch fibers are optimized for endurance activities, relying on aerobic metabolism and being fatigue-resistant. On the other hand, fast-twitch fibers are optimized for rapid, high-intensity activities, relying on anaerobic metabolism but getting fatigued more rapidly (Aleksandrowicz & Strączkowski, 2023; Kamunde et al., 2023; Frontera & Ochala, 2015).

Skeletal muscles can adapt and change in response to different environmental and physiological factors, including exercise. This adaptation occurs through mechanisms such as hypertrophy (an increase in muscle fiber size)**,** increased mitochondrial density (more mitochondria per unit of muscle, enhancing oxidative capacity), fiber type switching (transitioning between slow-twitch and fast-twitch characteristics), and improved metabolic flexibility (Ashcroft et al., 2024).

Skeletal muscle cells utilize a variety of sources to supply skeletal muscle energy requirements, including carbohydrates, lipids, and ketone bodies that are circulating in the bloodstream. Along with these circulating energy sources, skeletal muscle also relies on its stored energy reserves to meet the demands of muscle contraction (X. Li et al., 2019). Among the lipid categories, myocytes store intramuscular triglycerides as

triglycerides (TGs) in their protoplasm, serving as an important energy source for muscle contractions. The important role and complex interaction of skeletal muscle with larger metabolic systems emphasize the adaptability and efficiency of the body's musculoskeletal system (Watt & Cheng, 2017).

2.1.1 Intramuscular Triglycerides Storage in Skeletal Muscle

Intramuscular triglycerides have an important effect on health, muscular function, and metabolism, indicating their fundamental involvement in physiological processes (Shaw et al., 2010). The breakdown of IMTG releases fatty acid molecules into the bloodstream, which regulates energy metabolism during exercise and supplies the energy needs of skeletal muscle (Watt & Cheng, 2017). These fatty acids are an important source of energy, helping muscles to continue prolonged physical activity (Jordy & Kiens, 2014). The close relationship between IMTG and muscle function includes aspects such as performance and endurance, especially during endurance or high-intensity activities where IMTG oxidation increases (Bergman et al., 2018).

In addition to its role during exercise, IMTG is essential for overall health, and an imbalance and irregular accumulation known as myosteatosis can lead to a range of metabolic diseases, including obesity and type 2 diabetes (Henin et al., 2023). Myosteatosis, characterized by fat accumulation in skeletal muscle tissue, plays a critical role in altering metabolic recruitment and reducing muscle tissue elasticity (Correa-de-Araujo et al., 2020). This accumulation can lead to the development of metabolic disorders such as prediabetes and diabetic conditions, which can impair glucose tolerance, particularly in individuals who follow a sedentary lifestyle (Dlamini & Khathi, 2023). Myosteatosis is not only associated with metabolic disorders but also with decreased muscle performance and strength (Henin et al., 2023). Researchers have shown a negative relationship between muscle strength, which refers to the muscle's ability to exert force, and levels of muscular fat metabolism, highlighting that weaker muscles may have altered fat-processing efficiency. This indicates the importance of considering myosteatosis not only as a potential cause of various metabolic disorders and impaired muscle performance but also as an indicator of disease (Borghi et al., 2022). It further supports the idea that

myosteatosis, the accumulation of fat within muscle tissue, negatively impacts muscle performance, which refers to the ability of muscles to generate force and sustain activity, and muscle function, encompassing energy production, movement efficiency, and maintenance of muscle size.

Understanding the impact of myosteatosis in people with sedentary lifestyles emphasizes the importance of exercise in reducing risk factors associated with fat accumulation in skeletal muscles. People can manage the risk factors associated with myosteatosis and improve their overall metabolic and musculoskeletal health through an active lifestyle (Ramírez-Vélez et al., 2021). Studying the effects of myosteatosis provides useful information about how fat stores and accumulates in muscles in different ways. It also expands our knowledge of metabolic disorders and potential treatments to improve muscle health and performance.

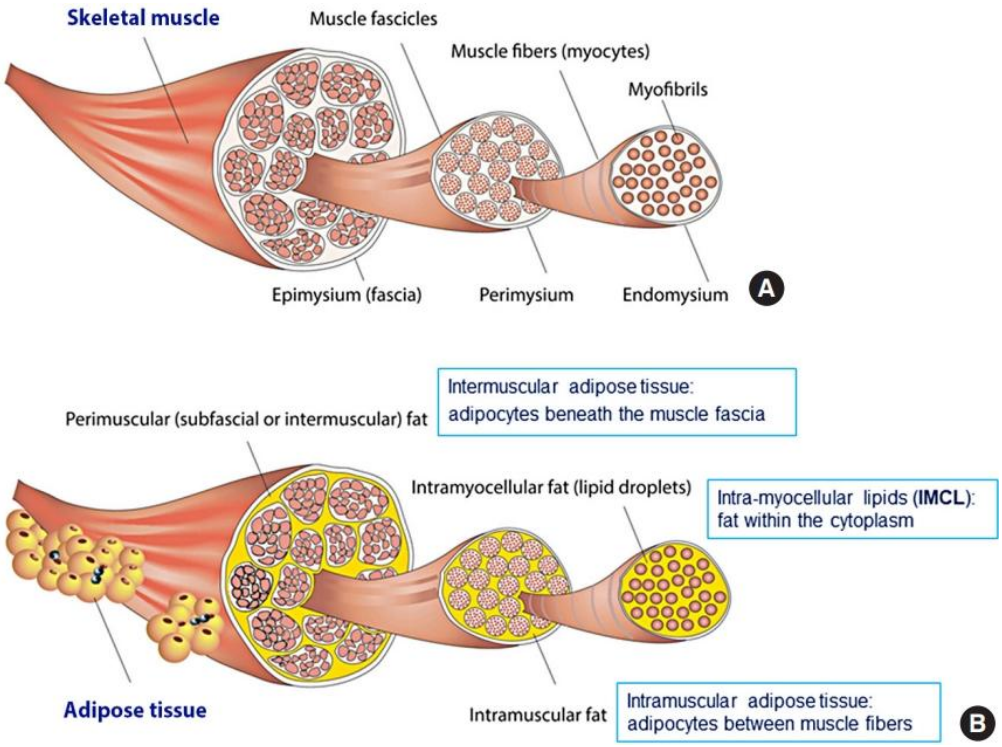

Figure 1: Skeletal Muscle and IMTG distribution

The adapted figure (H.-K. Kim & Kim, 2021) shows the anatomical composition of skeletal muscle and the distribution of adipose tissue within it. (A) Skeletal muscle consists of intramyocellular myofibrils, muscle fibers, and fascicles, which are

surrounded by thicker layers of connective tissue known as endomysium, perimysium, and epimysium. (B) Within skeletal muscle, fat can be categorized into intramyocellular and extramyocellular components.

Exercise not only influences skeletal muscle mobility but also has an important impact on physiological processes supporting muscle health and function. For example, it promotes angiogenesis, which is the formation of new blood vessels, improving oxygen and nutrient delivery to muscles. It also enhances neuromuscular coordination, which is the nervous system's and muscles' ability to work together efficiently, leading to better control and strength in skeletal muscle movements (Mulya et al., 2017; Huber-Abel et al., 2012). Exercise training also improves neuromuscular coordination, reduces energy expenditure, and increases movement efficiency (Lavin et al., 2019).

Malcolm A. West et al. conducted a prospective cohort analysis with 123 patients receiving pancreatic and hepatobiliary surgery. Notable results were observed regarding the influence of myosteatosis on aerobic physical fitness. The study strongly linked myosteatosis, not sarcopenia, to a reduction in the subjects' aerobic physical performance. Fat accumulation in skeletal muscle tissue is an important component in decreased aerobic physical fitness, emphasizing the importance of identifying and treating this factor in muscle structure (West et al., 2019). This points out exercise as an effective way to minimize the negative effects of myosteatosis on the body, supporting healthy muscular function and metabolic balance.

From this study, we can conclude that there is a deep connection between improved endurance, strength, and metabolic capacity, all of which contribute to optimal skeletal muscle function. Enhanced endurance allows muscles to continue physical activity for longer periods; increased strength boosts force production; and better metabolic capacity improves skeletal muscles’ efficiency in generating and using energy. Thus, obesity and metabolic disorders caused by an inactive lifestyle can impair processes such as IMTG metabolism, lipid accumulation, and mitochondrial function. However, a phenomenon known as the "athlete paradox" shows that higher levels of intramuscular triglycerides in endurance athletes do not necessarily indicate weak metabolic health. High IMTG levels

typically link to conditions like insulin resistance and type 2 diabetes, where muscle fat accumulation impairs muscular metabolic function. However, trained athletes efficiently use these fat stores as a quick, accessible energy source during prolonged exercise. The athlete paradox suggests that regular endurance training adapts how muscles store and process fat, making IMTG a beneficial fuel rather than a risk factor. PGC-1α signaling, a key pathway that enhances fat metabolism and energy efficiency in muscle cells, may influence this phenomenon. In the following sections, we will explore this phenomenon in more detail.

2.1.2 IMTG and the Athlete's Paradox

The phenomenon known as the athlete's paradox describes how endurance-trained athletes have increased insulin sensitivity and oxidative capability even though their intramuscular triglyceride levels are high, similar to those of those with insulin resistance (Wolins & Mittendorfer, 2018). The contradictory effects of exercise training and metabolic dysregulation on the amount and type of intramyocellular triglycerides (IMTG) stored in muscle may explain the athlete's paradox. Exercise improves both the storage and efficiency of IMTG usage in muscle cells, which improves metabolic function. Conversely, metabolic dysregulation, a condition associated with diseases such as insulin resistance, can lead to the accumulation of IMTG, disrupting normal muscle metabolism. These variables interact with other metabolic pathways, affecting muscle performance and health (X. Li et al., 2019; Wolins & Mittendorfer, 2018). Although the molecular mechanisms behind the athlete's paradox remain incompletely understood, various factors, such as the size and distribution of IMTG droplets, can influence it (Daemen et al., 2018; Gaspar et al., 2021; Gemmink et al., 2017, 2021).

Lipid-metabolizing enzymes, specifically known as lipases, significantly influence the metabolism of intramuscular triglycerides. These include key enzymes such as adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), which are important in breaking down triglycerides stored in skeletal muscle tissue. In addition to their primary role in lipid breakdown, these enzymes help mitochondria function by regulating the availability and absorption of different fatty acid subcategories: short-, medium-, and

long-chain fatty acids (Abdelhalim, 2024; Alsted et al., 2013; Meex et al., 2015; Olivecrona, 2010; Schweiger et al., 2006). They also control the growth and shape of mitochondria, showing how important they are to IMTG metabolism and possibly explaining things like the "athlete's paradox," where trained muscles store and use lipids efficiently. Physical exercise training affects all these factors, including intensity, duration, frequency, dietary condition, and genetic background, all of which contribute to this paradox (De Geus, 2021).

As mentioned, endurance training can improve skeletal muscle's ability to store and utilize IMTG. In addition to changes in lipase activity and expression, this improvement may also be caused by increasing mitochondrial density (mitochondrial biogenesis) and oxidative capacity. This is possible because PGC-1α and other related transcription factors in its signaling pathways, such as AMP-activated protein kinase (Cheng et al., 2018a; Qian et al., 2024), become more active. PGC-1α is a crucial protein that regulates mitochondrial function, especially in the case of energy production for cellular activities, by influencing how well our mitochondria operate and determining their preferred energy sources. This regulation has significant implications for metabolic health, as efficient mitochondrial function is essential for energy production. In athletes, fat droplets in muscle cells provide a ready energy source during exercise, while in sedentary individuals, these droplets can negatively impact mitochondrial function and lead to health issues. PGC-1α could change the composition of IMTG droplets by stimulating the storage of more easily oxidized fatty acids, such as medium-chain triglycerides, compared to long-chain triglycerides (Supruniuk et al., 2017). Oxidative metabolism benefits directly from these increased mitochondrial numbers and efficiency, which are beneficial for better cellular performance and endurance function. Due to its modified oxidative capacity, the muscle efficiently generates ATP from various nutrients, including fats, carbohydrates, proteins, and fatty acids derived from intramyocellular triglycerides (Kolodziej & O'Halloran, 2021). Moreover, increased mitochondrial efficiency can reduce the production of reactive oxygen species (ROS), harmful byproducts of energy production, and enhance cellular signaling processes (S. Huang et al., 2016). Exercise-induced ROS at moderate levels appear to be involved in initiating additional

metabolic adaptations; however, excessive ROS can be harmful to the body (F. He et al., 2016).

Regular physical exercise is an excellent way to reduce myosteatosis, and different studies have shown that the release of epinephrine during physical training and the contractions of isolated muscles result in intramyocellular triacylglycerol lipolysis in the skeletal muscles of humans and rodents (Peters et al., 1998; Roepstorff et al., 2004a). Another recognized mechanism in IMTG breakdown is the upregulation of PGC-1α and modulation of mitochondrial fatty acid oxidation involved in this process, as PGC-1α is one of the most important molecules essential for regulating mitochondrial biogenesis and skeletal muscle's metabolic changes in response to exercise (Iijima et al., 2023; Mulya et al., 2017; West et al., 2019). This metabolic change is beneficial for the overall health and function of muscle tissue in several ways, as it helps clear excess fat that may have accumulated within the muscle cells, reducing the risk of inflammation, and improving muscle performance. Regular exercise boosts activation of PGC-1α, enhancing mitochondrial function by increasing mitochondrial quantity and quality, which improves energy generation efficiency in muscle cells. This alteration improves the reliance on fatty acid oxidation, allowing muscle cells to more effectively utilize stored lipids for energy production. The body enhances its ability to process and metabolize intramuscular fat, thereby preventing fat accumulation in muscle tissues, referred to as myosteatosis (Ramírez-Vélez et al., 2021).

2.2 Mitochondria: Powerhouses of Cellular Energy

Similarly to other tissue cells, there are many cellular organelles, such as mitochondria, in the cytoplasm of muscle cells (Elhanany-Tamir et al., 2012), which is the main focus of our study. Mitochondria are double-membraned organelles (Fig. 2) present in the cells of many species, including humans. Often referred to as the "powerhouse" of the cell, mitochondria account for approximately 40% of the total volume within muscle cells and play a vital role in energy production by generating around 95% of the cell's ATP via oxidative phosphorylation (L. Chen et al., 2022; Gottlieb et al., 2021; B. Zhou & Tian,

2018). This organelle primarily produces energy through thermodynamic reactions (Mitchell & Moyle, 1967).

The discovery of the TCA cycle in the 1950s was the starting point of research interest in mitochondria's role in energy production. The electron transport chain of mitochondria, which consists of four complexes: I, II, III, and IV (Figure. 2), plays a crucial role in the production of ATP, the body's primary energy source (Giacomello et al., 2020). However, it is important to note that the function of mitochondria extends beyond energy production. Research conducted after the 1990s has shown that this organelle influences processes such as apoptotic cell death, various signaling pathways, autophagy regulation, stem cell differentiation, and immune response modification (Giacomello et al., 2020; Gomes et al., 2011; X. Liu et al., 1996; Scorrano et al., 2002).

Many cellular components connect and interact closely with mitochondria, forming an active network necessary for cellular metabolism. As an example, we can mention its connection with the endoplasmic reticulum (ER), which is one of the most researched organelles residing inside the cell (Ronayne & Latorre-Muro, 2024).

Numerous studies, particularly after Holoszy's pioneering research, have investigated the effects of different types of exercise on mitochondrial density and function across age groups. Holoszy’s findings demonstrated that exercise training significantly increases mitochondrial density and respiratory enzyme activity in skeletal muscle, improving aerobic work capacity (Holloszy, 1967).

For instance, one study examined the impact of age and exercise training on various aspects of skeletal muscle in horses. Ten elderly horses and eight young horses comprised the subjects. Researchers measured muscle fiber type (which affects endurance and power), satellite cell abundance (important for muscle repair and growth), mitochondrial density (the number of mitochondria in muscle cells), mitochondrial function (how well energy is made), and oxidative capacity. Samples of muscle from the triceps brachii and gluteus medius were taken at weeks 0, 8, and 12 of exercise training. The results indicated that the mitochondrial density, function, and oxidative capability of older horses were essentially lower than those of younger horses. However, both age groups showed

increased mitochondrial density and function in response to exercise training (Latham et al., 2021).

In their study, Koltai et al. used Wistar rats to investigate how exercise training affects mitochondrial biogenesis and protein quality control in skeletal muscle, including running on a treadmill at 60% of initial VO_2max. They evaluated several characteristics, including the Lon protease 1 (LONP1), SIRT1 activity, AMPK, pAMPK, PGC1-α, UCP3, and mitochondrial mass indicators. Their findings showed that exercise training had a beneficial effect, either decreasing or correcting age-related declines in mitochondrial parameters (Koltai et al., 2012).

2.2.1 Mitochondrial morphology

All of the mitochondrial function depends on its special morphology, particularly the double-layered structure (Giacomello et al., 2020). The structure and arrangement of mitochondria vary between tissues according to factors such as the cell cycle and metabolic or cellular signals that affect their functions, including ATP synthesis (Friedman & Nunnari, 2014). They are not only different in terms of energy production but also in ROS production according to the shapes of mitochondria. For example, fragmented mitochondria produce higher amounts of ROS and can be removed more effectively via the mitophagy procedure (Friedman & Nunnari, 2014). While higher levels of ROS can be harmful, they also play important roles as signaling molecules in different cellular processes. This difference is visible in the dimensions, roundness, and structure of mitochondrial membranes (Giacomello et al., 2020).

Mitochondria are highly dynamic organelles that constantly change their shape and structure, a phenomenon known as "mitochondrial dynamics." This includes fission, where a single mitochondrion divides into two, and fusion, where two mitochondria merge into one. These processes are essential for maintaining mitochondrial quality and adapting to the cell's changing needs. Factors such as nutrient availability and physical activity can significantly influence these dynamics (Giacomello et al., 2020). A more detailed explanation of mitochondrial dynamics is included in the mitochondrial dynamics section of this dissertation. The change in morphology greatly affects their

function, especially their ability to produce energy. This concept will be explained in more detail in a separate section of this dissertation.

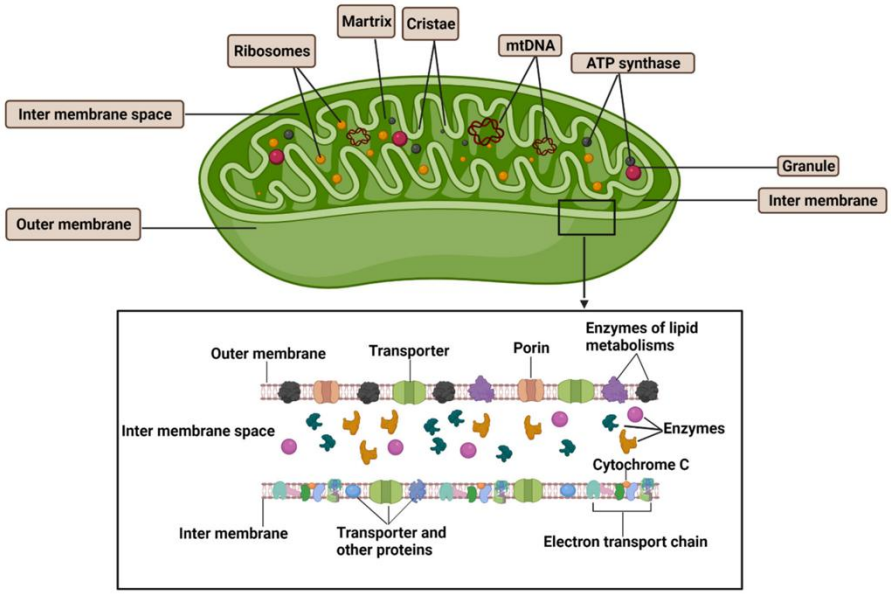

Figure 2. Structure of a Mitochondrion

This adapted figure (X. Zhang et al., 2021) illustrates the essential components of a mitochondrion, the cell's powerhouse. The inner and outer membranes, which have an intermembrane gap, are emphasized.

Mitochondria are defined by a unique double-membrane structure essential for their function (Schiaffarino et al., 2022). The outer mitochondrial membrane (OMM) acts as a selective barrier, containing channels that permit the passage of small molecules (under 5,000 Da) between the cytoplasm and the mitochondrion (Giacomello et al., 2020). The OMM contains channels that facilitate the entry of ions, ATP, ADP, and other molecules between the cytoplasm and mitochondrial matrix (Giacomello et al., 2020; Lefkimmiatis et al., 2013).

The OMM contains channels that facilitate the entry of ions, ATP, ADP, and other molecules between the cytoplasm and mitochondria matrix (Giacomello et al., 2020; Vercellino & Sazanov, 2022).

High-resolution imaging methods, such as high-resolution confocal laser scanning microscopy and wide-field fluorescence microscopy, which are known for their ability to capture large fields of view and provide high-speed imaging of dynamic processes, have facilitated the identification of the intermembrane space (IMS), an important membrane of the mitochondria (Adam et al., 2021; Alexander et al., 2021; Dumitru et al., 2021). Through the use of these advanced imaging techniques, researchers have discovered several important transporters located in the IMS between the OMM and the IMM that play crucial roles in communication with the cytosol and with other mitochondrial parts for the uptake of different metabolites, lipids, or metal ions, as well as in the regulation and execution of apoptosis (Backes & Herrmann, 2017; Herrmann & Riemer, 2010). The strategic location of these transporters within the IMS significantly impacts cellular communication and the control of apoptosis, influencing the release of pro-apoptotic factors.

This membrane creates a fluid-filled space accompanied by porins in the OMM and is the first region to receive diffused ions and small proteins playing functions in cellular activity by facilitating enzymes to phosphorylate other nucleotides and proteins by using ATP from the matrix (Patergnani et al., 2021). OMM porins, which work like channels, transport the generated pyruvate from glycolysis and fatty acids to the IMS, which is later converted into acetyl CoA for starting TCA cycle reactions within the IMM (Endo & Sakaue, 2019). The respiratory chain complexes responsible for oxidative phosphorylation are located in the IMM and actively transfer protons from the IMM to the IMS (Vercellino & Sazanov, 2022). Recent improvements in imaging technologies have made it easier to study mitochondrial morphology in more detail. Understanding the complex structure of mitochondria and the arrangement of transporters within the various mitochondrial membranes is crucial. Because they make it easier for pro-apoptotic factors to be released, the arrangement of these transporters has a big effect on how cells communicate and how apoptosis is controlled. Understanding this is important for coming up with new ways to treat diseases related to mitochondria since mitochondrial dysfunction is linked to many conditions, such as metabolic syndromes and neurodegenerative disorders (Chu et al., 2022). Further research into the role of PGC-1α

in regulating mitochondrial membrane composition, transporter activity, and overall mitochondrial dynamics may offer novel therapeutic targets for these conditions.

2.2.2 Mitochondria and Fatty Acid Oxidation

When it comes to exercise, especially at low and moderate intensities, lipids are recognized as a crucial energy source (Jordy & Kiens, 2014; Muscella et al., 2020b). Exercise-induced fatty acid (FA) oxidation is the process by which fatty acids are broken down to produce energy during physical activity. It is affected by IMTG lipolysis in adipose tissue, the delivery of FA to the working muscle, the control of FA transmembrane transport in muscle cells, and mitochondrial metabolism (Muscella et al., 2020b; Shaw et al., 2010).

During long period of exercise, the breakdown of stored fat in adipose tissue and within muscles (IMTG) is controlled by muscle contractions and hormonal factors. The main hormonal mechanism for activating this pathway involves the phosphorylation of HSL and the removal of perilipin, allowing access to lipid droplets (Gemmink et al., 2021; Laurens et al., 2016). Muscle HSL activity is stimulated through contractile-based mechanisms, namely HSL phosphorylation via MAPK and PKC enzymes through ERK (Donsmark et al., 2003). Furthermore, endurance training induces adaptive responses that enhance the capacity for fatty acid oxidation. These adaptations include increased activities of beta-oxidation enzymes, the TCA cycle, and the electron transport system. Additionally, the transport of long-chain fatty acids into mitochondria via carnitine palmitoyl transferase (CPT) is enhanced, with muscle carnitine content playing a regulatory role (Muscella et al., 2020c).

Following lipolysis, released glycerol and fatty acids enter the bloodstream. Fatty acids, bound to albumin for transport, are delivered to various tissues, including muscle and liver. Within muscle cells, fatty acids are taken up via specific transporters, such as FAT/CD36 and FABPpm, before being transported into the mitochondria. Inside the mitochondria, fatty acids undergo beta-oxidation, a cyclical process that breaks down fatty acids into acetyl-CoA molecules (Aon et al., 2014). This process generates reducing

equivalents (NADH and FADH2) that fuel the electron transport chain, ultimately leading to ATP production.

Beta-oxidation is a highly efficient pathway for ATP regeneration but requires significant oxygen. The acetyl-CoA generated from beta-oxidation enters the TCA cycle, further contributing to energy production. The close interplay between lipolysis and mitochondrial function is essential for sustaining energy provision during exercise. PGC-1α, a master regulator of mitochondrial biogenesis and function, likely plays a role in coordinating these adaptations to exercise, potentially by influencing the expression of enzymes involved in fatty acid transport and oxidation (Sousa et al., 2018).

2.2.3 Mitochondrial reactive oxygen species

Oxidative stress occurs when there is an imbalance between the generation of reactive oxygen species (ROS) and the cell's capacity to neutralize these reactive molecules, significantly influencing cellular function and adaptation (Szechyńska-Hebda et al., 2022). This is the condition that occurs due to a restricted supply of oxidizing substances, with the primary ROS being hydrogen peroxide (H_2O_2), a non-radical compound, and superoxide anion ($O_2^{\bullet-}$), an extremely reactive free radical. Furthermore, oxidative reactions also involve the hydroxyl radical (HO•) and singlet oxygen $^1O_2$, which is a non-free radical compound (Jomova et al., 2023).

The main enzymatic activities that produce hydrogen peroxide (H2O2) include the following: Flavin-dependent oxidases and xanthine oxidases are present in the endoplasmic reticulum, peroxisomes, and cytosol; acyl-coenzyme A (acyl-CoA) oxidases are found in peroxisomes and are primarily involved in the oxidation of fatty acids; superoxide dismutase enzymes are found in mitochondria, the nucleus, peroxisomes, and the extracellular matrix (Demidchik, 2015; Exposito-Rodriguez et al., 2017; Hasanuzzaman et al., 2020; Smirnoff & Arnaud, 2019).

These ROS are produced from different cell organelles and are affected by internal and external factors. They have the ability to enter the cytosol and start to change patterns of gene expression in the cell nucleus through different signaling pathways to change

transcription factors (Choudhury et al., 2017; Szechyńska-Hebda et al., 2022). For example, in different cell types, excessive ROS production can result in the downregulation or reduction of mitochondrial biogenesis (Bouchez & Devin, 2019).

The effects of exercise on mitochondrial ROS production are important for understanding how exercise-induced oxidative stress interacts with muscle function and general health. When muscles contract during exercise, ROS is produced, and mitochondria are the primary endogenous source of it (Bouchez & Devin, 2019). Low-level ROS generation is induced by regular or appropriate exercise, which is beneficial for sustaining muscular function. On the other hand, high ROS brought on by intense or brief exercise can cause exhaustion, injury, and oxidative stress in the muscles (Szechyńska-Hebda et al., 2022).

Under intensive and endurance exercise conditions, malfunction of the mitochondrial respiratory chain can lead to oxidative damage to proteins, lipids, nucleic acids, and other parts of the cell (Demidchik, 2015; Jomova et al., 2023), leading to enhanced succinate-involved oxidative phosphorylation and improved oxidative stress caused by moderate or long-term regular exercise is strongly associated with muscle adaptation, highlighting the complex relationship between exercise and ROS (Powers et al., 2020).

The adverse effects of high amounts of free radicals from intensive physical activity on general health and muscular performance underline the significance of antioxidant systems in maintaining a balance of ROS levels during exercise. The antioxidant enzymes found in mitochondria are essential for reducing oxidative stress and preserving cellular integrity (F. Wang et al., 2021). Exercise significantly improves mitochondrial oxidative capacity in different diseases. For instance, patients with cardiovascular diseases (CVD) did not have healthy mitochondria.

The interplay between exercise, ROS production, and mitochondrial adaptation is complex and likely regulated by key factors such as PGC-1α. For instance, Brandt et al. used both PGC-1α knockout mice and wild-type mice as controls to investigate the effects of PGC-1α on muscle adaptations to different exercise intensities. These mice were given different intensities of acute (one session) or chronic (5 weeks) exercise regimens. Mice ran on treadmills for 40 minutes at low intensity or 20 minutes at moderate intensity

during the acute sessions. Twice-daily low-intensity (40 minutes) or moderate-intensity (20 minutes) sessions were part of the chronic training. Following exercise, muscle samples obtained at key intervals were meticulously analyzed by the researchers. This study demonstrated that the intensity of exercise influences the response of autophagy, a cellular recycling mechanism. Specific autophagy markers, LC3I and LC3II, showed delayed increases in response to intense exercise. An autophagy marker (LC3II) was similarly increased by prolonged training; however, this alteration seemed unaffected by the mice's training volume or intensity. PGC-1α-deficient mice exhibited different reactions to autophagy-associated proteins and indicators, such as p62. The degree of exertion seems to affect even PGC-1α's response to exercise (Brandt et al., 2017).

This suggests that PGC-1α overexpression and endurance exercise can improve the balance of ROS production. Further research into how PGC-1α regulates ROS balance during exercise and how this relates to IMTG metabolism and the athlete's paradox could provide valuable insights for optimizing exercise interventions for both health and performance.

2.2.4 SDHA and Citrate Synthase Key Players in Mitochondrial Energy Metabolism

Succinate dehydrogenase (SDH) and citrate synthase (CS) are two crucial enzymes involved in mitochondrial energy metabolism, specifically within the citric acid cycle (also known as the Krebs cycle). The SDHA gene expresses Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA), a protein that plays a crucial role in energy metabolism (Rasheed & Tarjan, 2018). It is one of the four subunits of the succinate dehydrogenase (SDH) enzyme, which is involved in energy generation in mitochondria and plays important roles in cellular energy metabolism, maintaining mitochondrial health, regulating ROS levels, and influencing cellular signaling pathways (Chattopadhyay et al., 2019; Zhao et al., 2017).

SDHA is involved in the oxidation of succinate to fumarate in the citric acid cycle, an important step in oxidative phosphorylation that facilitates ATP synthesis, the primary source of cellular energy (Nazar et al., 2019; Rutter et al., 2010). It also reduces

ubiquinone (coenzyme Q) in the aerobic electron transfer chain, contributing to the generation of ATP by oxidative phosphorylation (Rutter et al., 2010).

On top of the role it plays in energy metabolism, SDHA has other roles in maintaining the health of mitochondria and regulating cellular homeostasis, including modulating oxidative stress via controlling ROS production inside the mitochondria (Burgener et al., 2019). Similar to all the other mitochondrial proteins, PGC-1α modulates the transcription of this metabolic gene as well (S. Kong et al., 2022a). This connection shows SDHA's importance to PGC-1α regulatory pathways and healthy mitochondria.

Furthermore, the positive effects of exercise on SDHA expression and activity have been well documented. Studies suggest that exercise induces the upregulation of different molecules, including PGC-1α, consequently enhancing the expression of genes associated with mitochondrial biogenesis and function, such as SDHA (Hwang et al., 2022). These exercise-induced improvements in the expression of SDHA show the importance of physical activity in enhancing cellular resilience and metabolic efficiency.

A study by Bellar et al. on mice with tumors who have been treated with chemotherapy showed that moderate-intensity exercise helps increase the amount and function of mitochondria and reduces the removal of damaged mitochondria. This improvement in response to exercise was partially due to increased levels of certain proteins in mice with tumors that were treated with chemotherapy, as shown through western blot analysis by researchers (Ballarò et al., 2019).

Citrate synthase is an enzyme found in nearly all living cells that acts as a regulator in the first step of the citric acid cycle. It is found in eukaryotic cells' mitochondrial matrix, which is encoded by nuclear DNA rather than mitochondrial (H. Morrison, 2021). The citrate cycle functions as a kind of intermediate source through the synthesis of amino acids necessary for ketogenesis, lipogenesis, and gluconeogenesis, and it is also an important part of the electron transport chain (Wiegand & Remington, 1986). CS catalyzes the chemical reaction of acetyl coenzyme A's two-carbon acetate residue with a molecule of four-carbon oxaloacetate to generate the six-carbon citrate (Chhimpa et al., 2023).

On top of being a rate-limiting enzyme for the TCA cycle, this enzyme has important roles in enhancing the ETC energy cycle and mitochondrial health (Chhimpa et al., 2023). Moreover, CS serves as a quantitative indicator of mitochondrial integrity, function, mass, and respiratory chain enzymes. Reduced levels of CS lead to decreased citrate, cytoplasmic acetyl-CoA, and ACh neurotransmitter production, release, and resynthesis, resulting in a poor energy environment within the cells (Yubero et al., 2016). Reduced CS activity damages the aerobic ATP generation pathway and diminishes citrate or cytoplasmic acetyl-CoA (Alhindi et al., 2019).

PGC1α and citrate synthase (CS) activity are essential for controlling cells' energy metabolism. PGC1α directly influences the expression and activity of CS in several tissues (Austin & St-Pierre, 2012). Understanding this connection suggests potential therapies for metabolic diseases and methods to enhance cells' energy utilization. Furthermore, it illustrates how PGC1α regulates crucial mitochondrial enzymes, revealing the complex mechanisms that govern cellular energy metabolism (Austin & St-Pierre, 2012; Sumi et al., 2022).

The study by Fernandes et al. investigated the effect of aerobic activity training on male OB/OB mice. Fernandes et al. divided the eight-week-old mice into two groups of seven: the training and the sedentary groups. During an 8-week aerobic exercise training, the animals in the training group ran at 60% of their maximal speed. Pre-test evaluations revealed no significant differences between groups. Nevertheless, after the training program, the trained group showed a significant increase in skeletal muscle citrate synthase activity and improved running capacity. These modifications suggest enhanced oxidative metabolism and higher endurance. Further examination showed that the trained mice expressed more PGC-1α and CS genes on top of higher enzyme citrate synthase activity. This supports the observation that the exercise intervention increased the oxidative metabolism (Fernandes et al., 2020).

The coordinated regulation of SDHA and CS by PGC-1α and their upregulation by exercise highlights the importance of these enzymes in mitochondrial energy metabolism

and adaptation to physical activity. Further research is needed into how these enzymes contribute to IMTG utilization during exercise and how they are influenced by PGC-1α.

2.3 Lipid metabolism

Cellular energy stores are generally found in the form of triacylglycerol (TAG) within lipid droplets. The mobilization of these energy sources happens through lipolysis, a process involving the sequential breaking down of TAG (Althaher, 2022; Grabner et al., 2021; Lass et al., 2011). In the 1970s, researchers identified three main lipases that control the lipolysis pathway and are not attached to fatty acids: ATGL (adipose triglyceride lipase), HSL (hormone-sensitive lipase), and MGL (monoacylglycerol lipase) (Grabner et al., 2021; Lass et al., 2011). In Figure 3, the process of lipolysis and the release of free fatty acids from lipid droplets are illustrated. This process is started by the enzyme ATGL, which breaks down triglycerides into diglycerides (DAG) and releases one free fatty acid. Subsequently, HSL breaks down DAG to monoacylglycerol (MAG) and another FFA. Finally, monoglyceride lipase (MGL) further metabolizes MAG, producing glycerol and additional free fatty acids. The release of energy from stored triglycerides through this process is essential for supporting metabolic activities (Muscella et al., 2020c).

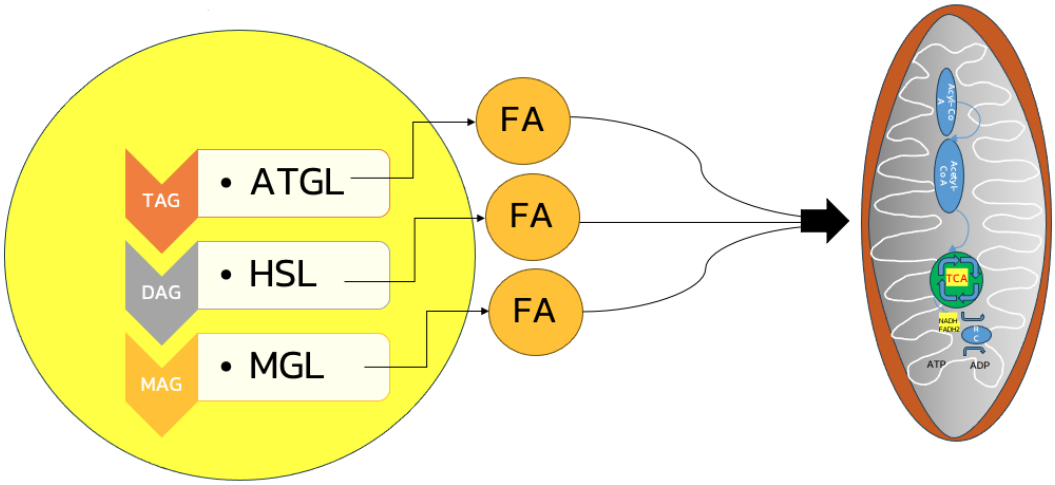

Figure 3 Schematic representation of lipolysis reactions within a lipid droplet in the cytosol illustrates the breakdown of stored triglycerides into free fatty acids and glycerol. Triglycerides (TAG) are sequentially hydrolyzed by adipose triglyceride

lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL). ATGL initiates the process, converting TAG to diacylglycerol (DAG) and releasing a free fatty acid (FFA). HSL then converts DAG to monoacylglycerol (MAG), releasing another FFA. Finally, MGL hydrolyzes MAG to produce glycerol and a third FFA. Transporting the released FFAs to the mitochondria for beta-oxidation provides energy for cellular activities.

2.3.1 ATGL: Initiating Lipolysis for Fatty Acid Release

Fats are stored in the body primarily as triglycerides within adipose tissue, as well as in intermuscular and intramuscular depots. These triglycerides serve as a crucial energy reserve, particularly during fasting, daily activities, and aerobic exercise (Chait & Den Hartigh, 2020; Muscella et al., 2020c). To utilize this stored energy, triglycerides must undergo lipolysis, the enzymatic breakdown into fatty acids and glycerol. Adipose triglyceride lipase (ATGL) plays a critical role in initiating this process (Grabner et al., 2021).

ATGL, sometimes termed PNPLA2 or desnutrin, plays an important role in the lipolysis process, acting as the first catalyst of the reaction, breaking down triglycerides into diacylglycerols (DAGs), the molecule that contains two fatty acid chains covalently bonded to a glycerol molecule through ester bonds and free fatty acid (Cerk et al., 2017; Prajapati et al., 2012). This enzymatic activity initiates the release of the energy stored in triglycerides (Cerk et al., 2017). Studies have shown that ATGL plays an important role in lipolysis in adipose tissue and other tissues such as the liver and skeletal muscle. In these tissues, ATGL contributes to lipid breakdown and influences energy metabolism, promoting a shift toward increased fat utilization. This function of ATGL supports the energy requirements of these tissues by providing fatty acids as fuel, which can reduce their dependency on glucose and help balance their energy sources (Zechner et al., 2009).

For instance, in skeletal muscle, ATGL deficiency leads to increased glucose uptake, suggesting a compensatory mechanism to meet energy demands (Schreiber et al., 2019). Another metabolic factor that is affected by ATGL is insulin, which is evidenced to be secreted by β-cells. Pancreatic ATGL helps to maintain glucose homeostasis,

emphasizing its function in overall health by regulating lipid and glucose metabolism (Trites & Clugston, 2019).

Studies using transgenic mice with either ATGL overexpression or deletion have provided interesting results, especially in tissue-specific models intended to understand its impact on glucose tolerance and insulin sensitivity. In the case of gene deficiency, mice showed different effects, including impaired TG lipolysis during fasting, increased TG synthesis, decreased cold tolerance, and increased insulin sensitivity (J. Li et al., 2023; Kienesberger et al., 2009; Haemmerle et al., 2006; Schoiswohl et al., 2015). Further examination of these animals showed a decrease in energy expenditure and reduced exercise performance, in line with decreased PPAR-α and -γ target gene activity in WAT, BAT, and liver (Ahmadian et al., 2009). Inhibition of ATGL results in different metabolical changes, including lipid accumulation, reduced oxygen consumption, and decreased fatty acid β-oxidation, which results in impaired metabolic balance and decreased body function (Han et al., 2021).

2.3.2 HSL: Hormone-Sensitive Lipase in Lipid Metabolism

Hormone-sensitive lipase (HSL), which is sometimes referred to as cholesteryl ester hydrolase (CEH), is an intracellular enzyme that plays an important role in lipid metabolism, especially in adipocytes, by breaking down TG molecules (Althaher, 2022). The term "Hormone-sensitive lipase" originates from research findings showing that its neutral lipase activity is sensitive to epinephrine hormone-induced lipolytic activity in adipose tissue. The name indicates its reactivity to hormones like catecholamines, ACTH, and glucagon, which stimulate the physiological function of this intracellular neutral lipase (Kraemer & Shen, 2002). HSL has a wide substrate specificity, including hydrolysis of triacylglycerol, diacylglycerol, 1(3) monoacylglycerol, cholesteryl esters, lipoidal esters of steroid hormones, retinyl esters, and water-soluble butyrate substrates in adipose tissue (Figure 3).

The feature that makes HSL different from other lipases like ATGL is that its enzyme activity against triacylglycerol and cholesteryl ester substrates is regulated by reversible phosphorylation, which allows for dynamic regulation of its reaction by adding or

removing phosphate groups from this enzyme, thereby changing its structure and function accordingly (Recazens et al., 2021). Yet, hydrolytic activity against diacylglycerol, monoacylglycerol, and water-soluble substrates is unaffected by phosphorylation. This 83Kda molecule is also expressed in muscle tissues, and its activity is affected by catecholamines. Thus, both phosphorylation-dephosphorylation and allosteric processes regulate HSL activity in adipose tissue and skeletal muscle. HSL is essential for mobilizing fat stored in different tissues, including subcutaneous and intermuscular ones, and can hydrolyze various lipid compounds (Watt et al., 2006). However, its main function is triglyceride breakdown, collaborating with ATGL during lipolysis. Research involving the inhibition of ATGL, as mentioned in the previous section, is the key enzyme in TG breakdown, increased fat accumulation, reduced oxygen consumption, and decreased fatty acid β-oxidation (Althaher, 2022; Han et al., 2021). Unexpectedly, when ATGL is blocked, protein expressions of total and phosphorylated HSL increase, but overall free fatty acid and lipase activity decrease (Han et al., 2021; Marvyn et al., 2015).

There has been a lot of research studying the effects of exercise on lipolysis, HSL expression, and function. Some studies show that exercise minimizes the activity or amount of HSL in muscle; however, physical training has improved HSL sensitivity to catecholamines in intraabdominal muscular tissue. HIIT triggers a significant increase in HSL phosphorylation in subcutaneous fat, resulting in greater post-exercise lipolysis than MICT. Liu et al. examined whether HIIT and MICT impact fat removal via HSL activity. To create obesity, they fed a high-fat diet to female C57Bl/6 mice. The mice were divided into three groups: one for acute exercise (one training session, evaluated at rest and at 0, 1, and 12 hours after exercise), one for long-term training (12 weeks of training combined with a 12-hour fast), and one for in vitro training (12 weeks of training plus fat cell activation and isolation). The running distances for the HIIT and MICT exercise interventions were the same. A major conclusion of the analysis was that a single HIIT session increased HSL activation in subcutaneous fat for at least 12 hours, an effect not seen after MICT. However, the analysis did not show a significant interaction between exercise type and time for total effects. Furthermore, visceral fat became more sensitive to signals of fat breakdown after long-term HIIT training. These findings suggest that the

benefits of HIIT for fat reduction come from increased fat mobilization over time, especially concerning visceral fat that is hard to break down, as well as from prolonged fat burning following exercises (Y. Liu et al., 2020).

2.3.3 Muscular Receptors of Short-chain Fatty (GPR41 and GPR43)

In lipid metabolism research, we come across various fatty acids, including short-, medium-, and long-chain fatty acids (SCFA, MCFA, and LCFA, respectively) (Metzler-Zebeli et al., 2021). Recently, SCFA has become the most common research topic, catching the attention of many researchers. This is because bacteria in the gut microbiota break down carbohydrates that our bodies can't digest without oxygen, leaving behind major metabolic waste products (D. J. Morrison & Preston, 2016). This produced SCFAs in the gut later enter the bloodstream and affect the physiology, body's health, and metabolism at many different levels, including the cellular, tissue, and organ levels (Lange et al., 2023). Mechanisms associated with gut barrier function, including glucose homeostasis maintenance, immunomodulation, and obesity regulation, mediate these effects (Chambers et al., 2018). Acetate (C2), propionate (C3), and butyrate (C4) are the most predominant SCFAs in the human body, as well as the colon (Portincasa et al., 2022). The designations C2, C3, and C4 denote the number of carbon atoms contained in SCFA (De Leeuw et al., 2019). As mentioned above, these fatty acids are necessary for a variety of physiological activities, including energy metabolism, gastrointestinal health, and immunological function. For example, Devadder et al. discovered in 2014 that butyrate and SCFA can considerably enhance glucose homeostasis (De Vadder et al., 2014).

The findings highlight butyrate's potential therapeutic role in glucose regulation, offering light on its positive benefits for metabolic balance. SCFAs support the general health and function of the gut, as well as the growth and development of intestinal epithelial cells (IECs), by acting as essential energy sources for these cells (Parada Venegas et al., 2019). Moreover, SCFAs promote the growth and activity of beneficial intestinal bacteria, which results in a balanced gut microbiota that improves immunological and intestinal health (Fusco et al., 2023). SCFAs can affect skeletal muscle metabolism through certain

receptors, in addition to supporting the immune system via their signaling pathways and gastrointestinal health (Abdelhalim, 2024; J. He et al., 2020).

The way SCFAs work in skeletal muscle metabolism is through specific receptors, like GPR41 (FFAR3) and GPR43 (FFAR2), which are GPCRs found in skeletal muscle cells (Gizard et al., 2020). When these receptors are activated by SCFA, PYY and glucagon-like peptide 1 (GLP-1) are released. This stimulation influences several aspects of muscle function, both directly and indirectly, as shown in Figure 4 (Larraufie et al., 2018). SCFAs bind to GPR41 and GPR43 in muscle cells, which has direct effects on how energy is used, glucose absorption, and other cellular processes. They also have indirect effects on muscle health through their effects on the gut hormones PYY and GLP-1 (Christiansen et al., 2018; den Besten et al., 2013). SCFAs also influence these hormones, regulating appetite, insulin sensitivity, and overall metabolic balance (Christiansen et al., 2018). They thereby indirectly affect the condition and functionality of the skeletal muscle system. SCFAs enter into the mitochondria of muscle cells, where they are used in the process of beta-oxidation 8/19/2025 1:21:00 PM to produce Acetyl-CoA molecules, and they subsequently enter the TCA cycle and start the following reactions (Martínez-Reyes & Chandel, 2020).

As previously mentioned, SCFAs function as signaling molecules due to their ability to bind to G-protein-coupled receptors such as GPR41/FFAR3 and GPR43/FFAR2, activating these receptors and subsequently influencing cell functions (Carretta et al., 2021). FFAR2 (GPR43) and FFAR3 (GPR41), related to each other by 52% similarity and 43% common features, influence the chemical processes of glucose and lipid metabolism (Frost et al., 2014; Swaminath et al., 2010 Christiansen et al., 2018). Kimura's research group discovered that GPR41, one of the SCFA receptors, SCFAs, and ketone bodies, controls SNS function in the regulation of mammalian energy homeostasis (Kimura et al., 2011). A Chinese research team investigated whether genes matching human GPR41 and GPR43 mediate pig short-SCFA regulatory effects. The findings showed that the GPR41 and GPR43 genes are located on pig chromosome 6. These genes exhibited tissue-specific and time-dependent expression in different pig tissues, including skeletal muscle (G. Li et al., 2014).

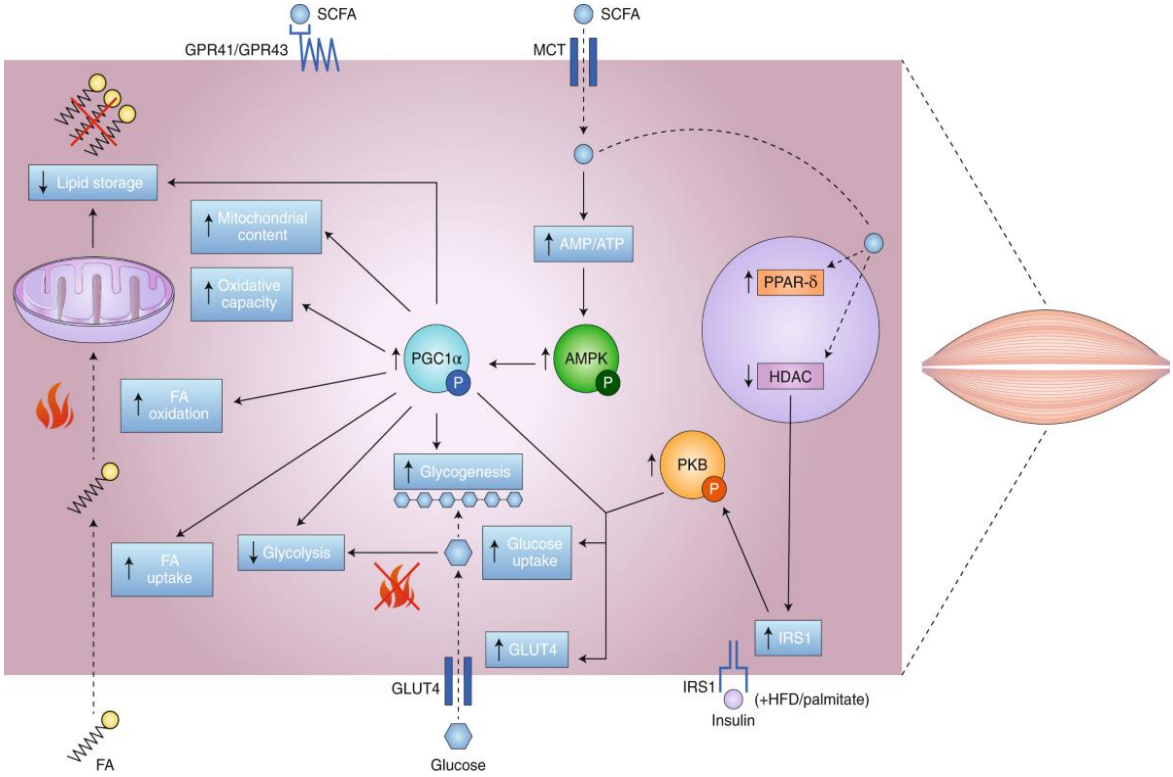

Figure 4: GPRs and AMPK activation

This figure, adapted from Frampton et al. (2020) illustrates the complex interplay between illustrates the complex interplay between GPR41/43, AMPK, PGC-1α, and mitochondria in regulating intramuscular lipid metabolism. Short-chain fatty acids (SCFAs) activate GPR41/43 receptors, triggering a signaling cascade that increases AMPK and PGC-1α (Frampton et al., 2020).

There is a possible, but mostly unknown, connection between PGC-1α-mediated metabolic adaptations and the SCFA-GPR41/43 axis. It's possible that SCFAs could alter PGC-1α, as shown in Figure 4. There is a possible, but mostly unknown, connection between PGC-1α-mediated metabolic adaptations and the SCFA-GPR41/43 axis. It's possible that SCFAs could alter PGC-1α expression or activity by activating GPR41/43. On the other hand, overexpression of PGC-1α may affect GPR41/43 receptor expression or sensitivity. Lu et al. found that giving mice SCFA supplements like acetate, propionate, butyrate, or mixtures of these substances increased the expressions of GPR43 and GPR41 in adipose tissue. This led to the breakdown of triglycerides and the oxidation of free fatty

acids to produce brown fat. It also decreased body weight in an HFD-fed mouse model, increased the expression of PGC-1α, activated AMPK, and made insulin work better (Z. Gao et al., 2009).

2.4 mTOR Signaling: Implications for Lipid Metabolism

The mammalian target of rapamycin (mTOR) is a 289-kDa protein kinase recognized as a key regulator and control center in our cells, essential for numerous processes, including cell growth, division, and metabolism. The way mTOR regulates these cellular processes can be influenced by environmental factors such as variations in endocrine signals, cytokine status, energy, and nutrient availability (Y. Gao & Tian, 2023; G. Y. Liu & Sabatini, 2020).

Over the past few years, scientists have conducted many studies to show connections between mTOR and lipid metabolism and how it can affect the storage, metabolism, and utilization of fats in the body. Many growth factors work in different ways to control the building and breaking down of skeletal muscle. One way they do this is by turning on the PI3K/Akt/mTOR pathway (Yoshida & Delafontaine, 2020). Activation of mTORC1 is associated with increased protein and lipid synthesis, which promote cellular growth (Gatica & Klionsky, 2017). Liu et al. researched to investigate the complex interplay between AMPK, mTOR, and S6K1, aiming to explain the underlying mechanisms contributing to exercise-induced insulin sensitization in skeletal muscle. The primary focus was to understand how exercise training could effectively reverse lipid-induced insulin resistance. Using a model of 34-week-old male wild-type C57BL/6 mice, the researchers applied a 6-week exercise training regimen on a motor-driven treadmill, with sessions conducted 5 days per week, gradually increasing in intensity and duration. Through methods such as western blotting, they examined lipid metabolism, signaling pathways, and skeletal muscle adaptations. They showed that exercise training significantly improved insulin sensitivity, activated AMPK, and modulated mTOR and S6K1, ultimately restoring lipid-induced insulin resistance (X. Liu et al., 2012).

2.5 PCYT2: A Key Enzyme in Lipid Metabolism and Homeostasis

Multiple cellular pathways are involved in lipid metabolism, breakdown, and biosynthesis. In previous sections we reviewed research and literature about lipid breakdown pathways, including lipolysis and beta-oxidation; however, it is also important to understand the processes involved in lipid biosynthesis, in which lipids are synthesized from smaller molecules. New phospholipids, such as phosphatidylethanolamine (PE) and phosphatidylcholine (PC), are made through the Kennedy pathway. These phospholipids are important parts of cell and mitochondrial membranes (Pavlovic & Bakovic, 2013).

Phosphoethanolamine cytidylyltransferase (PCYT2) is an important enzyme for controlling the Kennedy pathway (Gibellini & Smith, 2010). PCYT2 helps in the change of CTP and phosphoethanolamine into CDP-ethanolamine, which is an important step in making phosphatidylethanolamine (Cikes et al., 2022; Wellner et al., 2013). PE, which is a structural part of cell membranes, is also very important for keeping the integrity of both cell membranes and mitochondrial membranes (Cikes et al., 2022).

PCYT2 is an important enzyme for the structure of the membrane and significantly affects mitochondrial function, including oxidative phosphorylation, ATP generation, and how muscle tissue controls its energy balance. Research indicates that low levels of PCYT2 disrupt energy balance in muscle tissues and adversely affect mitochondrial activity. The removal of PCYT2 in skeletal muscle negatively affects mitochondrial function and energy balance, as demonstrated by our group in collaboration with Austrian researchers in 2023 (Cikes et al., 2023). The study indicates that while PCYT2 and PGC-1α are important for optimal cellular function and metabolism, it does not establish a direct link between the two.

2.6 PGC-1*α*: Master Regulator of Skeletal Muscle Metabolism

Physical exercise induces both morphological and biochemical changes in skeletal muscle, as it does in many other body tissues. These changes include increased mitochondrial density, enhanced oxidative capacity, improved glucose uptake, greater lipid oxidation, and optimized substrate utilization in skeletal muscle tissue (Figure 3).

According to Besseiche et al. (2015), these adaptations are largely driven by the activation of the transcription factor PGC-1α (Besseiche et al., 2015). PGC-1α, or Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1 Alpha, was initially identified as a coactivator for peroxisome proliferator-activated receptor gamma (PPAR-γ) in brown adipose tissue (Puigserver et al., 1998). This coactivator is highly expressed in energy-demanding tissues, including skeletal muscle, and can be significantly upregulated in response to physiological stimuli such as cold exposure and physical activity (Liang & Ward, 2006a; Vega et al., 2015a). In skeletal muscle, PGC-1α promotes mitochondrial biogenesis and fatty acid oxidation, enhancing endurance and energy efficiency during prolonged exercise (Liang et al., 2009).

PGC-1α and different coactivators have an important function in regulating various physiological processes. It regulates energy homeostasis by balancing energy intake and expenditure, thereby ensuring cellular health and functionality (Figure 3). PGC-1α also affects mitochondrial density, or the total number of mitochondria in every cell. Regular physical activity improves this number and helps the cells for better energy generation. PGC-1α also involves oxidative phosphorylation (OXPHOS), an essential process in mitochondria that generates ATP, the primary energy source for cells, through oxygen-based use. Especially during aerobic exercise, this mechanism plays a crucial role in continuous energy production by efficiently utilizing oxygen to metabolize fats and carbohydrates, thereby fueling muscles. PGC-1α also increases muscle endurance during prolonged, low-intensity workouts by helping fatty acid oxidation—the breakdown of lipids to produce energy (Cheng et al., 2018a).

In animal studies, researchers have utilized different genetic models to investigate the direct impact of PGC-1α on skeletal muscle function and exercise adaptation. These models include whole-body overexpression or knockout of the PGC-1α gene, as well as targeted overexpression or knockout in specific muscle tissues. For example, studies overexpressing PGC-1α and some of its isoforms (PGC-1α-b) in skeletal muscle have shown higher expression of it in the skeletal muscle tissue and improvements in endurance function and fatigue resistance, highlighting the critical role of PGC-1α in regulating muscle function during exercise (Hatazawa et al., 2015a; Karlsson et al., 2021;

Tadaishi et al., 2011a). These observations support the idea that increasing PGC-1α levels through genetic manipulation could enhance endurance performance by elevating oxidative capacity, the muscle's ability to generate energy through aerobic (oxygen-dependent) processes, which is crucial for prolonged physical activity and promoting mitochondrial biogenesis, the process of creating new mitochondria within cells. This increase in mitochondria provides more "power plants" within muscle cells to produce energy, leading to improved endurance and resistance to fatigue.

2.6.1 PGC-1α and Regulation of Mitochondrial Biogenesis

Mitochondria are essential for determining overall energy levels, as they generate ATP, the primary source of cellular energy, through different mechanisms, including oxidative phosphorylation and deacetylation by sirtuin1 (Radak et al., 2020). The term "mitochondrial biogenesis" refers to the process of synthesizing new, healthy mitochondria to meet the energy requirements of biological activities and to regenerate defective or malfunctioning mitochondria (Popov, 2020). It has been shown that PGC-1α is very important for controlling mitochondrial biogenesis through transcriptional processes, which leads to more mitochondria in the cell (Lim et al., 2022). Different stressors, such as starvation, low oxygen levels (hypoxia), oxidative stress, cytokine profiles, or working out, can activate PGC-1α (Abu Shelbayeh et al., 2023; Lim et al., 2022; Suntar et al., 2020). It is essential to ensure that mitochondria continue to operate properly to preserve skeletal muscle mass (Z. Xu et al., 2022), mainly because any dysfunctions in mitochondria result in the activation of catabolic signaling pathways, which increase the activation of genes associated with muscle wasting and dysfunction (Romanello & Sandri, 2021).

2.6.2 PGC-1α and the Pathways affect mitochondrial function

Complex molecular interactions allow skeletal muscle to adapt dynamically to different signals, especially during exercise (McGee & Hargreaves, 2020). Mihaylov et al. (2023) suggested that PGC-1α is one of the most important factors in keeping mitochondria and energy metabolism balanced (Mihaylov et al., 2023). PGC-1α is directly responsible for the increase in mitochondrial biogenesis in response to exercise as an outside stimulus. It

does this by connecting to specific nuclear target gene promoters (Islam et al., 2018). PGC-1α exhibits responsiveness to extracellular stimuli and intracellular metabolic conditions. Important regulators such as SIRT1/3, TFAM, and AMPK affect its responses (Abu Shelbayeh et al., 2023; Cantó & Auwerx, 2009). Numerous studies have also confirmed that PGC-1α plays a role in increasing the expression of genes involved in mitochondrial respiration (Finck, 2006). This coactivator also modulates the expression of genes involved in fat metabolism and fatty acid oxidation, including those regulating the TCA cycle and mitochondrial fatty acid oxidation (FAO) pathway (Hatazawa et al., 2015a). One important aspect of PGC-1α is that it can increase peroxisomal activity, which includes breaking down long- and very-long-chain fatty acids (T.-Y. Huang et al., 2017).

Many research projects use the animal model in which the PGC-1α gene is overexpressed in the whole body or skeletal muscle to investigate the different roles of overexpression of PGC-1α. One way is to use transgenic mice (MCK-PGC-1α) with more PGC-1α than the normal amount under the muscle creatinine kinase promoter. This method enabled researchers to study the exact role of the muscular PGC-1α pathway on changes during exercise in different tissues (J. Lin et al., 2002). In their study, Brandt and colleagues examined the relationship between PGC-1α and exercise intensity-dependent adaptations. The researchers used whole-body PGC-1α knockout (KO) mice and wild-type (WT) animals, subjecting them to both acute and chronic exercise treatments. When compared to chronic exercise, which consisted of a five-week training schedule with either low-intensity training (LIT) or moderate-intensity training (MIT) sessions twice daily, acute exercise consisted of a single bout of treadmill jogging at low intensity (for forty minutes) and moderate intensity (for twenty minutes). They collected quadriceps muscle samples immediately after exercise, three and six hours into recovery for acute exercise, and before and after the training period for analysis of the exercise. These samples were acquired to analyze physical activity. The intensity of the exercise determined increases in the autophagy marker p62 in skeletal muscle during acute exercise.

Furthermore, PGC-1α needed to be present to regulate the proteins involved in autophagy. The results of the exercise training showed that PGC-1α was necessary for the alterations

in LC3I and LC3II protein levels that were caused by the training intervention. Furthermore, regardless of the degree of exercise training, there was a rise in LC3II. On the other hand, the lack of PGC-1α affected the p62 protein concentration. Furthermore, it was discovered that the mRNA responses of PGC-1α isoforms associated with exercise were intensity-dependent. In conclusion, the findings of this study demonstrate that the intensity of exercise has a distinct impact on the autophagy and mitochondrial function markers found in the skeletal muscle of exercise groups (Brandt et al., 2017).

2.6.3 PGC-1α - AMPK - Irisin Pathway

The AMPK/PGC-1α axis has an important role in regulating mitochondrial energy metabolism and maintaining cellular energy balance (Figure 4). The AMPK molecule consists of three subunits as follows: α (63 kDa), β (38 kDa), and γ (38 kDa). Among these, the α subunit plays a role in the catabolic pathways (Willows et al., 2017). Changes in the intracellular AMP/ATP ratio had a significant effect on AMPK activity. When the levels of AMP and ATP increase, AMPK is activated (González et al., 2020; Grahame Hardie, 2016; Hardie, 2007). AMPK-α activation suppresses anabolic reactions, preventing the use of the ATP (D. Garcia & Shaw, 2017). However, prolonged stress might negatively affect AMPK expression. Reduced AMPK levels in cells result in reduced PGC-1α's effectiveness and negatively impact mitochondrial function (S. Kong et al., 2022b).

Recent studies show that when muscles contract, they release chemicals called myokines. These help skeletal muscles communicate with other organs like adipose tissue, bone, liver, kidneys, and the brain (Huh, 2018a). One notable myokine is irisin, derived from the cleavage of fibronectin type III domain-containing protein 5 (FNDC5). (Huh, 2018b; Huh et al., 2012, 2014). In mice with muscular PGC-1α overexpression, PGC-1α triggers the production of FNDC5 protein (Boström et al., 2012). Exercise releases irisin into the blood by breaking down the FNDC5 (Huh et al., 2014). This leads to increased energy use in subcutaneous fat tissue through the activation of browning in fat cells. Moreover, irisin enhances energy expenditure, exerts anti-inflammatory effects, and improves mitochondrial function (Jo & Song, 2021; Lei et al., 2024).

Numerous studies have examined the effects of various exercise types on the PGC1-α-AMPKα and irisin pathways. One study randomized untrained males aged 19–28 to evaluate their performance either in functional resistance training (FRT) with 4-5 sets of 20 repetitions maximum (RM) at 40% 1RM or traditional resistance training (TRT) with 4-5 sets of 12 RM at 70% 1RM, three days a week for six weeks. The study examined the effects of acute and chronic resistance training at different intensities on molecular responses and their relationship to muscular fitness in these young men; key findings indicated significant increases in lean body mass, a decrease in body fat percentage and fat mass in the FRT group, and improvements in muscular fitness variables in both groups. Serum levels of AMPK, PGC-1α, irisin, and insulin-like growth factor-1 increased significantly in both groups, while myostatin decreased significantly after acute and chronic training in both groups. This research demonstrates that both short-term and long-term resistance training can alter molecular responses through the AMPK/PGC-1α/irisin signaling pathway, leading to stronger and more functional muscles (Huh, 2018; Huh et al., 2012). According to Lin et al. (2020), swimming activates the AMPK/SIRT1/ PGC-1α axis, highlighting the role of exercise-driven mitochondrial activation in enhancing the health and stability of muscle tissue and neurons. All of the aforementioned studies give important additional insights on the molecular processes behind the positive effects of exercise on muscle performance and mitochondrial health (J.-Y. Lin et al., 2020).

2.6.3.1 AMPK Activation and its Role in Lipid Metabolism

According to Kim et al. (2016), the oxidation of FA in different tissues, including the liver and muscle, is significantly mediated by the AMPK (Roepstorff et al., 2004b).

AMPK plays a crucial role in regulating HSL activity; phosphorylation of HSL increases its enzymatic activity, enabling it to break down TAGs into free fatty acids and glycerol efficiently (J. Kim et al., 2016; S.-J. Kim et al., 2016).

When AMPK was knocked off in male C57BL/6 mice fed a high-fat diet, mitochondrial fuel oxidation went up and lipogenesis decreased. An increase in the AMP/ATP ratio triggered AMPK activation, leading to these effects. As a result, the activation of AMPK

improved the metabolic health of mice by reducing fat storage and increasing insulin sensitivity, thereby protecting the liver tissue from cancer. This research highlights the potential benefits of focusing on AMPK to address metabolic issues related to lipid homeostasis (T. H. Kim et al., 2013). Based on these studies, it appears that AMPK has the potential to limit the production of cholesterol and fatty acids, reduce lipid formation, and promote fatty acid oxidation by activating specific proteins further down the line.

Beyond its impact on lipolysis, AMPK significantly influences lipogenesis by suppressing the activity of mTOR complex 1 (mTORC1), a key regulator of cell growth and metabolism. Under conditions of cellular energy depletion, such as hypoxia, AMPK activity increases due to a decrease in ATP production and a corresponding rise in the AMP/ATP ratio. This activation of AMPK leads to the inhibition of mTORC1, ultimately suppressing lipogenesis (González et al., 2020) (González et al., 2020).

2.6.4 PGC-1α and Nitric Oxide Synthase (eNOS)

Nitric oxide (NO) synthase (NOS) enzymes, specifically endothelial NOS (eNOS) and neuronal NOS (nNOS), are necessary for the production of NO, which is involved in skeletal muscle metabolism and other physiological activities (Gantner et al., 2020).

ENOS is involved in many physiological processes and facilitates the synthesis of NO, which promotes the conversion of L-arginine into NO by human endothelial cells, increasing vasodilation and blood circulation (V. Garcia & Sessa, 2019; Oliveira-Paula et al., 2016). Ojami et al. (2010) conducted experiments on dogs with type 1 diabetes and showed that this metabolic disorder reduces nitric oxide (NO) bioavailability due to increased oxidative stress. They also observed significant changes in the expression of genes associated with oxidative stress and mitochondrial function, emphasizing the vital role of NO in supporting cardiac health in diabetic conditions (Ojaimi et al., 2010). eNOS affects blood vessel function and helps in maintaining the appropriate ratio of aerobic to anaerobic energy metabolism by improving mitochondrial activity, which preserves muscle health (Leung & Shi, 2022). Increased eNOS activity during exercise promotes angiogenesis and the formation of new blood vessels, which helps meet the higher oxygen and nutrient demands of muscles during physical activity. The increased production of

NO as a result of eNOS activation enhances blood flow to muscles, speeding up recovery and improving muscular endurance by ensuring a steady supply of oxygen and nutrients needed for better exercise performance (Padilla et al., 2011; Ross et al., 2023). Continuous eNOS activation is necessary to maintain skeletal muscle, physical fitness, and performance, as well as appropriate blood flow and metabolic balance and a positive reaction to exercise (Momken et al., 2004). Many research studies have shown interactions between different NOS types and the PGC-1α gene. As previously pointed out, PGC-1α plays a critical role in controlling the synthesis and function of mitochondria and is associated with regulating NOS activity. According to research, higher PGC-1α levels have been linked to eNOS phosphorylation, which is necessary for eNOS activation and NO production. Exercise effectively activates PGC-1α, highlighting the complex interaction between exercise, PGC-1α, and eNOS (Gantner et al. ,2020). Furthermore, studies have linked eNOS activation to regulating oxidative damage resulting from physical exertion.

In the study by Kinugawa et al. (2005), they evaluated the exercise capacity of heterozygous manganese superoxide dismutase (SOD2) knockout mice (SOD2+/−) using a treadmill test. This research group linked decreased levels of eNOS expression to a reduction in exercise capacity; they proposed that higher amounts of mitochondrial superoxide anion (O2−) inactivate NO, elevating $\dot{V}o2$ and decreasing exercise capacity (Kinugawa et al., 2005). The dual role that eNOS plays in both muscle and vascular function highlights how crucial it is to the physiological alterations needed for improved exercise performance and overall health. The function of PGC-1α in maintaining vascular health was investigated by Craige et al (Craige et al., 2016). These findings show the importance of NO in exercise performance and highlight the potential role of PGC1α in reducing mitochondrial dysfunction and maintaining exercise capacity.

PGC-1α's impact goes beyond eNOS to potentially include neuronal nitric oxide synthase (nNOS) in skeletal muscle. eNOS and nNOS play critical roles in NO synthesis, which is essential for muscle metabolism and blood flow regulation (Baldelli et al., 2014). Specifically, nNOS plays a significant role in sympatholytic, the process by which the effects of the sympathetic nervous system are reduced or inhibited. Through this

regulation, NO helps balance the body's 'fight or flight' response, which is crucial for maintaining optimal muscle function during exercise (Hirai et al., 2018). Current studies suggest that PGC-1α can regulate both the activity and expression of nNOS, despite the incomplete understanding of the exact mechanisms of the PGC-1α-nNOS relationship. It is known that NO is important for muscle function; it acts as a signaling molecule that affects processes like controlling blood flow, taking in glucose, initiating mitochondrial energy production, and muscle contraction, which needs further exploration (Huber-Abel et al., 2012; Moon et al., 2017).

Exercise as a stimulator of PGC-1α has the potential to indirectly affect nNOS activity via this mechanism. Calcium/calmodulin and AMPK can both activate eNOS and nNOS, which is important to know because they play a role in controlling PGC-1α (Huber-Abel et al., 2012). They studied the effects of decreased endothelial PGC-1α on vascular function using a combination of in vitro and in vivo techniques, such as cell culture experiments, RT-PCR analysis of mRNA expression, blood pressure monitoring, protein assessment using Western blotting, and direct measurement of nitric oxide (NO•) bioavailability. Their results showed that the PGC-1α EC KO mice experienced significantly increased blood pressure, indicating hypertension. Furthermore, these mice showed obvious indicators of vascular dysfunction, including a decreased response to stimuli and poor dilation of blood vessels. Lower nitric oxide bioavailability, which is an important vasodilator promoting blood vessel relaxation and improving circulation, was associated with these results in transgenic animals (Huber-Abel et al., 2012). Additionally, there was a decrease in the production of eNOS. They further clarified the chemical mechanism underlying these observations. They showed that PGC-1α protects blood vessels by triggering the transcription factor that promotes eNOS production, estrogen-related receptor alpha (ERRα). In the end, this PGC-1α/ERRα/eNOS pathway sustains healthy vascular function and stimulates NO production. The contribution of Craige et al.'s study to our knowledge of PGC-1α's function in vascular health is substantial. According to their research, appropriate expression of endothelium PGC-1α is essential in preventing cardiovascular disorders linked to endothelial dysfunction and hypertension. Additionally, it emphasizes the therapeutic potential of focusing on the

PGC-1α/ERRα/eNOS pathway to prevent and treat cardiovascular disease (Craige et al., 2016).

2.7 PGC-1α and Sirtuins

PGC-1α and the Sirtuin family are two major players in cellular metabolism. They produce a complex network of molecular interactions that regulate different metabolic pathways. The important transcriptional coactivator PGC-1α works with sirtuins, a group of enzymes that have deacetylase activity dependent on NAD+, to change many cellular functions that are not related to making energy. Discovering the intricate relationship between PGC-1α and sirtuins takes us to the core of cellular regulation, where these masters of metabolism lead a series of actions that determine how a cell reacts to alterations in energy requirements, oxidative stress, and general homeostasis (Radak et al., 2020).

2.7.1 PGC-1α and Sirt1

Sirtuin 1 (Sirt1), also known as the silent mating type information regulation 2 homolog 1 protein, is a type III deacetylase enzyme from the sirtuin family that plays a key role in regulating cellular metabolism (DiNicolantonio et al., 2022). Sirt1 functions by removing acetyl groups from various proteins, such as histones and transcription factors (Yue et al., 2016), thereby controlling the activity of key regulators like PGC-1α. Through the deacetylation of PGC-1α, Sirt1 modulates its activity, influencing downstream pathways such as lipid metabolism (Khan et al., 2015). As it was mentioned earlier, PGC-1's activity and functional role are precisely controlled by several changes, including ubiquitination, methylation, acetylation, and phosphorylation (Fernandez-Marcos & Auwerx, 2011).

SIRT1 plays a crucial role in controlling the transcription of the PGC-1α gene, which shows its physiological importance (Amat et al., 2009). Exercise has been shown to affect the Sirt1/PGC-1α pathway in skeletal muscle. For example, in one study using an animal model, Huang et al. examined the effects of a 12-week swimming protocol on the SIRT1/PGC-1α pathway in rats of different ages. The study utilized male Sprague-Dawley rats of varying ages (3, 12, and 18 months) and divided them into groups based

on their exercise levels: exercise and passive control. Following the 12-week program, the researchers assessed body composition, muscle characteristics, and the levels of certain proteins in both the gastrocnemius and soleus muscles. In all trained groups, the SIRT1, PGC-1α, and AMPK levels were higher in both muscle types when compared to the passive controls. These results suggest that swimming might change the expression of proteins in the soleus muscles of older rats, especially those that are connected to the SIRT1/PGC-1α pathway (C.-C. Huang et al., 2016).

Koltai et al. investigated how exercise affects SIRT1 activity in male Wistar rats, both young (3 months old) and elderly (26 months old). They separated the rats into two age groups, with each group further divided into control and exercise subgroups. The training routine consisted of a 6-week treadmill running program that progressively increased intensity to 60% of the rats' VO2 max. They used a modified procedure to ensure safety for older rats by adjusting the intensity and duration to avoid injuries based on their physical state. Their data indicated that exercise boosted SIRT1 activity in both age groups. The researchers proved SIRT1's involvement as a deacetylase by establishing a direct relationship between enhanced SIRT1 activity and decreased protein acetylation. They found that exercise has the potential to increase SIRT1 activity, which may help slow down some parts of the aging process (Koltai et al., 2010).

2.7.2 PGC-1α and Sirt3

Sirtuin 3 (SIRT3) is an important member of the NAD+-dependent deacetylase family. It is mostly found in mitochondria and plays roles in both the stress response and cellular metabolism. By deacetylating essential proteins, SIRT3 controls important energy metabolism processes like fatty acid metabolism, antioxidant defenses, and mitochondrial energy production (J. Zhang et al., 2020).

SIRT3 and PGC-1α are efficiently activated by exercise. Engaging in physical activity increases SIRT3 synthesis and PGC-1α activity. SIRT3 expression is elevated by PGC-1α, leading to a positive feedback loop that improves mitochondrial health and function (X. Kong et al., 2010). Moreover, SIRT3 is essential for controlling mitochondrial fatty acid oxidation, a crucial aspect of lipid metabolism. SIRT3 also deacetylates important

enzymes involved in fatty acid breakdown (Hirschey et al., 2010). Previous research on human subjects and animal models consistently shows that exercise has the ability to increase SIRT3 levels. This includes research examining both aerobic exercise and high-intensity interval training (HIIT), using both acute (single exercise session) and chronic (long-term training) interventions. Researchers have shown increased SIRT3 levels, specifically in skeletal muscle tissue, suggesting notable advantages for mitochondrial function and lipid metabolism (L. Zhou et al., 2022).

Vargas-Ortiz et al. (2015) investigated how a 12-week aerobic exercise program affected the levels of PGC-1α and SIRT3 in overweight teenagers. The participants consisted of 14 overweight or obese male adolescents, with an average age of 15.5 years, who followed the prescribed exercise program. The training involved 50-minute sessions, three times a week, with an intensity of 70–80% of their maximum heart rate. Body composition data and muscle biopsies were collected before and after the intervention to assess fat levels and measure the proteins SIRT3 and PGC-1α. They also evaluated aerobic capacity, specifically the VO2 peak. The measurements after the exercise intervention showed a significant increase in the expression of both SIRT3 and PGC-1α. In addition, the participants experienced a reduction in body fat percentage and waist size, as well as an improvement in their VO2 peak. The researchers found a correlation between SIRT3 and PGC-1α levels following the training program. Their research suggests that aerobic training alone has the potential to enhance SIRT3 and PGC-1α expression in sedentary, overweight, or obese adolescents. This indicates the potential metabolic benefits of exercise, regardless of any dietary changes (Vargas-Ortiz et al., 2015). The findings of these studies emphasize the crucial role of SIRT3 in cellular metabolism, its relationship with PGC-1α, and its involvement in intramuscular lipid metabolism. These results highlight the beneficial impact of aerobic exercise on SIRT3 expression and its potential implications for mitochondrial function and fatty acid oxidation.

2.8 PGC-1α and Mitochondrial Health and Dynamics

The LONP1 gene encodes the mitochondrial matrix protease LONP1, which is critical for maintaining mitochondrial protein quality. The primary function of this enzyme is to break down irregular or damaged proteins inside the mitochondria, maintaining the integrity as well as the function of the mitochondrial proteins' homeostasis, which is essential for the synthesis of cellular energy and general cellular health (Pollecker et al., 2021; Z. Xu et al., 2022; Zanini et al., 2023). Interestingly, LONP1 is not only essential for basic cell maintenance and function, but it also plays an essential role in controlling the synthesis of mitochondria and the cell's defense against oxidative damage (Zanini et al., 2023). The development of metabolic diseases, age-related diseases, and neurodegenerative disorders are all influenced by this protein (Bota & Davies, 2016). Although the importance of this protein is widely recognized, more research is required about the regulation and activation of LONP1 in mitochondria and exploring its interactions with other components or proteins within cells. PGC-1α, as the master regulator of mitochondrial biogenesis and cellular energy metabolism, has a significant influence on the expression of the mitochondrial protease LONP1 as one of its downstream targets (Jannig et al., 2022). PGC-1α can bind to and activate the promoter region of the LONP1 gene; thus, increased levels of PGC-1α result in increased expression of LONP1 (M. Chen et al., 2023).

Research indicates that exercise increases levels of LONP1 in aged animals' skeletal muscles, which improves mitochondrial quality control and potentially reduces age-related mitochondrial diseases (Zanini et al., 2023). For example, studies on mice have shown that long-term exercise enhances LONP1 protein expression, strengthening antioxidant defenses and improving overall health.

The variability and flexibility of mitochondria in terms of their appearance and location within cells are known as mitochondrial dynamics, which generally refers to the process that controls mitochondrial division and merging (Wai & Langer, 2016). This aspect of mitochondria is studied to understand how these organelles adapt to changing cellular conditions and contribute to overall cell function, since proper mitochondrial dynamics

are crucial for optimal cellular processes, including cell division, quality control, response to cellular stress, distribution among cells, and maintenance of cellular function and tissue development (Karbowski & Youle, 2003).

The two primary proteins involved in mitochondrial dynamics are Fis1 and Mfn1. Fis1, originally discovered in the outer mitochondrial membrane of yeast, plays a crucial role in regulating mitochondrial fission, which is essential for maintaining the network, shape, and function of mitochondria in cells. Additionally, Fis1 is involved in peroxisomal fission and human mitochondrial fission, highlighting its multifaceted importance in the cellular function (Wai & Langer, 2016; Wolf et al., 2020). Many cellular functions in our body depend on this protein since it serves multiple functions, including mitophagy and apoptotic pathways (Varuzhanyan et al., 2021). The crucial role that Fis1 plays in mitochondrial fission is by affecting the division of mitochondria into smaller pieces, which is controlled by combining the shape of the mitochondria with the energy level of the cell (Ihenacho et al., 2021a). Studies have shown that exercise elevates Fis1 levels, with treadmill running in mice demonstrating enhanced mitochondrial quality and reduced diabetes-induced cell death (Ihenacho et al., 2021b; Tolosa-Díaz et al., 2020).

Romanello et al. used adult male CD1 mice in a study that exposed the TA muscle to conditions that caused muscular atrophy. They evaluated mitochondrial function using several approaches, such as respirometry and enzyme activity tests. Their results showed the crucial role of mitochondrial fission and the remodeling of key mitochondrial proteins, particularly Fis1, in the progression of muscle atrophy (Romanello et al., 2010).

The Mfn1 gene encodes a protein called Mfn1, located in the outer mitochondrial membrane, which plays a crucial role in mitochondrial membrane adhesion and fusion (Tolosa-Díaz et al., 2020). Acting as a GTPase, Mfn1 facilitates the connection of mitochondria to form larger organelles through the fusion process. This process reshapes the composition of lipids, mitochondrial DNA, and proteins within the mitochondria, leading to the reorganization of essential cellular components and the enhancement of overall mitochondrial function (Moon & Jun, 2020; Yu et al., 2020). Many diseases in humans are linked to mitochondrial dysfunction and fragmentation, as well as

neurodegenerative disorders, metabolic syndromes, age-related diseases, and some cancers (Moon & Jun, 2020; Zhunina et al., 2021).

Campos et al. randomly used male Wistar rats that experienced myocardial infarction surgery or a comparable procedure without left anterior descending coronary artery ligation to be sedentary sham-treated (control), sedentary heart failure (HF), and exercised heart failure (HF-Ex) groups. The HF-Ex rats received treadmill training for 8 weeks, five days a week, for 60 minutes daily at 60% of their maximum aerobic capacity.

Following a 12-week exercise intervention, physiological parameter evaluations and biochemical analysis of cardiac structure, autophagy, mitochondrial dynamics, and function showed that exercise increased mitochondrial fusion markers, including MFN1. The increased levels of MFN1 in the exercise group were associated with fewer but larger myocardial mitochondria (Campos et al., 2017).

Fis1 and MFN1 work together to form a network that influences each other's functions, with their expression regulated by proteins like PGC-1α (Yu et al., 2020). PGC-1α, along with pathways such as the AMPK-SIRT1-PGC-1α axis, plays a key role in modulating their activity. Maintaining a balance between mitochondrial fission and fusion is essential for muscle health, as any disruption can result in muscle wasting and dysfunction (Ihenacho et al., 2021a; Tolosa-Díaz et al., 2020; Yu et al., 2020).

2.9 NAMPT/Visfatin A Multifaceted Regulator of Muscle and Metabolic Function

Nicotinamide phosphoribosyl transferase (NAMPT), also known as visfatin, is an enzyme that has many roles in cellular reactions, especially regarding muscular and mitochondrial activity (Dahl et al., 2012). Researchers first identified NAMPT as a pre-B cell colony-enhancing factor (PBEF) and later recognized its role in generating nicotinamide adenine dinucleotide (NAD+), an essential coenzyme for many muscular and cellular reactions (Garten et al., 2011). Studies in humans and rodents have shown that NAMPT affects muscle metabolism and performance by influencing glucose metabolism in this tissue and its sensitivity to insulin, which results in changes in the metabolic health of the skeletal muscle (Audrito et al., 2020; Basse et al., 2021). Also, NAMPT is closely related to

mitochondrial activity, ensuring mitochondrial integrity and bioenergetics (Goody & Henry, 2018).

The NAMPT metabolic pathway is linked to NAD-dependent sirtuin (SIRT) signaling, forming the NAMPT-NAD-SIRT cascade. According to Zhu et al., different organs have linked NAMPT to lipid metabolism control (Zhu et al., 2022). Studies show that NAMPT expression and activity affect lipid metabolism in different ways. Obesity, NAFLD, and insulin resistance are associated with higher levels of NAMPT, indicating its involvement in lipid dysregulation. Stromsdorfer et al. conducted a study using an animal model. They compared wild-type mice with transgenic mice overexpressing NAMPT in skeletal muscle (NamptTg) and muscle-specific NAMPT knockout mice (mNKO). Their findings revealed that mice lacking NAMPT in adipocytes exhibited severe insulin resistance in adipose tissue, liver, and skeletal muscle. They observed that mice lacking NAMPT in adipocytes showed insulin resistance across adipose tissue, liver, and skeletal muscle. They concluded that adipose tissue function and systemic insulin sensitivity are critically dependent on NAMPT-mediated NAD+ production in adipocytes. Additionally, impaired adipose tissue function was observed, characterized by altered plasma fatty acid concentrations and decreased adiponectin levels (Hagberg & Spalding, 2023).

As Wang et al. (2017) show, NAMPT affects lipid metabolism through different metabolic pathways, such as the Sirt1/AMPKα/SREBP1 signaling pathway. In their study, they focused on both animals and cell cultures to investigate the role of NAMPT in hepatic steatosis resulting from a high-fat diet (L.-F. Wang et al., 2017).

The complex pathways involved in skeletal muscle metabolic imbalances, such as inflammation and diabetes, are closely linked to the interaction between NAMPT and lipid metabolism, particularly the regulation of intermuscular adipose tissue. The connection between NAMPT and lipid metabolism, especially the control of intermuscular adipose tissue, is an important factor. Dysregulation of this factor can lead to metabolic imbalances in skeletal muscles, similar to diabetes and inflammation. Research has linked accumulated fat in the muscle to lower muscle function and insulin resistance. Also, many research studies have shown that NAMPT may play a role in

oxidative stress, which in turn affects lipid storage and utilization (Audrito et al., 2020; Zhu et al., 2022)

Research suggests an association between NAMPT, exercise, and PGC-1α expression. Recent research indicates that exercise boosts NAMPT expression, which affects PGC-1α gene expression and enhances mitochondrial function and metabolic health. NAD, a byproduct of NAMPT activity, also regulates energy metabolism, motility, temperature management, and antigen protection (Koh & Kim, 2021). High-energy molecules like creatine phosphate aid in the quick generation of ATP in skeletal muscle cells, which requires NAD. NAMPT has a crucial role in modulating mitochondrial function, modifying PGC1a activity, and influencing important processes across several tissues, according to recent studies (Song et al., 2017).

In the study by Koltai et al. on SIRT1 activity in aged rats (which was mentioned in the PGC-1α and sirt1 section), they also evaluated NAMPT activity changes in the rats of different ages in response to exercise. Exercise-induced increased relative activity of SIRT1 is probably a result of this increase in NAD+, the fuel for SIRT1. This finding supports the idea that SIRT1 and NAMPT are connected. It also suggests that exercise raises NAMPT levels, which in turn increases SIRT1, which affects PGC1a and other factors related to mitochondria (Koltai et al., 2010).

Further research is needed to fully understand the complex interplay between NAMPT, exercise, and PGC-1α in regulating muscle metabolism and mitochondrial function. Exploring whether exercise influences NAMPT expression or activity and how this might relate to IMTG metabolism, and the adaptations observed in athletes.

3. Objectives

This research was designed to explore the mechanisms of muscle-specific PGC-1α overexpression and exercise-related adaptation dependent on IMTG metabolism. The primary goal was to determine if the changes in IMTG metabolism are mainly caused by PGC-1α or affected by other exercise-related factors. PGC-1α is recognized for its established functions in mitochondrial biogenesis and fatty acid oxidation; however, its specific influence on IMTG metabolism is not fully understood.

3.1 Hypotheses

Based on the literature review and identified research gaps, we developed the following hypotheses:

Hypothesis 1: PGC-1α overexpression in skeletal muscle and endurance exercise training improve mitochondrial health and function markers, leading to enhanced endurance performance.

Hypothesis 2: PGC-1α overexpression in skeletal muscle and endurance exercise training will improve triglyceride breakdown markers, leading to increased reliance on fat as an energy source in the quadriceps muscle tissue of mice.

Hypothesis 3: PGC-1α overexpression in skeletal muscle and endurance exercise training modulate the expression of key enzymes involved in mitochondrial dynamics, contributing to improved metabolic flexibility and exercise adaptation in the quadriceps muscle tissue of mice.

Hypothesis 4: PGC-1α overexpression in skeletal muscle and endurance exercise training change key signaling pathways, such as the AMPK-α and sirtuin pathways, in the quadriceps muscle tissue of mice.

3.2 Relevance of the Research

The results of this study would improve our understanding of the molecular mechanisms underlying the adaptations of skeletal muscles induced by exercise. Understanding the

complex interplay between exercise, metabolism, and genetics will allow us to determine how PGC-1α regulates mitochondrial activity and intramuscular lipid metabolism. The findings have significant implications for developing therapeutic strategies for metabolic diseases such as obesity, type 2 diabetes, and cardiovascular disease.

4. Material and Methods

4.1 Animals: Housing, Study Groups, and Ethical Approval

In this study, we used 40 C57BL/6-Tg (Ckm-Ppargc1a)31Brsp/J mice. These mice, a transgenic strain, express the PGC-1α gene more specifically in their skeletal muscle, under the control of the mouse muscle creatine kinase promoter. This genetic modification leads to a change in muscle fiber composition, resulting in more type II oxidative phenotypes than normal wild-type mice. Such transgenic models are particularly valuable for investigating skeletal muscle physiology, exercise adaptations, oxidative capacity, and metabolic homeostasis. We selected male mice to reduce hormonal changes that could alter muscle metabolism, as female hormonal cycles might add additional considerations. All mice were ten months old, which is considered middle age for this strain. Middle age in C57BL/6-Tg mice ranges from 8 to 15 months, which is consistent with The Jackson Laboratory's definition of middle age as 10–14 months for these mice. To ensure the validity of our results, we randomly distributed the mice into four experimental groups while maintaining a balance in age to minimize the risk of selection bias by forming groups with similar baseline characteristics.

We compared physiological parameters in the presence and absence of PGC-1α overexpression using two sedentary control groups. The first group consisted of eleven mice with normal wild-type levels of PGC-1α (wt-C), while the second group included eleven mice with genetically induced overexpression of PGC-1α (PGC-1α-C).

Two additional groups of mice underwent an exercise program to investigate the effects of exercise. The PGC-1α exercise group (PGC-1α-Ex) comprised nine mice with high levels of PGC-1α who participated in the exercise program. Similarly, the wild-type exercise group (wt-Ex) included nine mice with normal levels of PGC-1α who also underwent the exercise program.

We kept all the animals in the animal house located in the Research Center for Molecular Exercise Science, Hungarian University of Sports Science in Budapest, which houses all of its mice in an environmentally controlled animal facility. Particular regulations for

temperature, humidity, light cycle (12 hours of light and dark), and ventilation were followed by this facility to guarantee the best possible circumstances for the care of the animals. We freely provided the mice with standard laboratory chow and water, ensuring their constant access to sustenance.

The National Animal Research Ethical Committee of Hungary approved ethical standards and criteria that were followed throughout all of the experimental processes. This study, approved by the ethical committee under approval number PE/EA/62-2/2021, guarantees that all animal handling, manipulations, and data collection procedures were carried out humanely and with the highest regard for the animal's welfare.

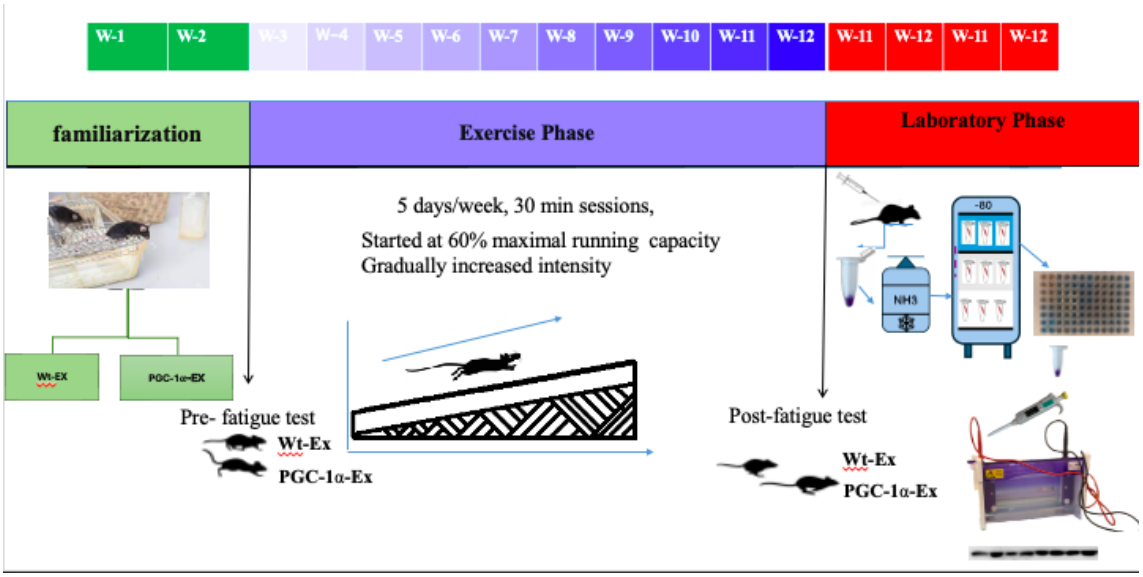

Figure 5 Study Timeline. The timeline illustrates the sequence of activities conducted over 14 weeks. In Week 1, animals underwent familiarization with the treadmill. This was followed by an exhaustion pre-test in the second week. From Weeks 3 to 12, regular training sessions were conducted. In Week 13, a post-training exhaustion test was performed, and animals were sacrificed. Tissues were flash-frozen in liquid nitrogen and stored at -80 °C for later analysis. Biochemical analyses began in Week 14.

The different phases of the study are color-coded: familiarization (green), training (blue), and biochemistry analysis (red).

4.2 Training Protocol

After allowing the mice in the training groups to become familiar with the treadmill, we performed a fatigue endurance test to practically evaluate their maximum running capacity, which is a key measure for assessing the animals' peak running ability as explained by (Dougherty et al., 2016) . Based on the data collected from the endurance test, we initiated the training at 60 percent of the animals' peak running capacity and progressively increased the exercise intensity on a weekly basis.

Training started at 60% of each mouse's maximum running capacity. The training continued for 10 weeks, with five days of 30-minute training sessions per week (Figure 6).

Figure 6 A Mouse Undergoing Endurance Exercise Testing.

The fatigue endurance test shows a mouse from the exercise intervention group running on a motorized treadmill. A gentle tail stimulus encourages the mouse to continue running until exhaustion, ensuring accurate determination of its maximal running capacity.

4.3 Western Blot Protocol

4.3.1 Tissue collection and homogenization:

Following accepted ethical standards, 48 hours after the last training session, we humanely put the mice to sleep to remove their quadriceps tissue. We removed the quadriceps muscles, a key muscle region involved in running and submerged them in liquid nitrogen to quickly freeze the tissue and maintain its metabolic integrity. We then kept the frozen muscle samples at -80 °C to ensure their quality and stability for further molecular analysis (Lei et al., 2024).

4.3.2 Tissue homogenization

We used an accurate weight scale to separate 50–80 grams of quadriceps muscle in the same way for all samples. We then mixed them by using a lysis buffer containing 137 mM NaCl, 20 mM Tris-HCl pH 8.0, 1% Nonidet P-40, 10% glycerol, plus protease SIGMAFASTTM Protease Inhibitor Tablets (S8820-20TAB) and PhosSTOPTM phosphatase (4906845001) inhibitor tablets to prevent degradation. We placed the muscle tissues in this lysis buffer within an ice container and then homogenized them using an Ultra Turrax homogenizer (IKA, Staufen im Breisgau, Germany).

4.3.3 Protein extraction and concentration determination

We utilized the Bradford assay to determine the protein concentration in the tissues, to ensure that all of the prepared sample contained an equal amount of protein for loading into polyacrylamide (SDS-PAGE) gels for Western blot analysis. To do this, we used Bio-Rad's protein assay dye reagent concentrate (#5000006) that had been diluted four times and the BCA protein concentration assay as a standard (Sigma A3059) to find out how much protein was in the mixed samples. We calibrated the SDS-PAGE Western blot loading amount by balancing the protein content of different samples based on the measured protein concentration (Lei et al., 2024).

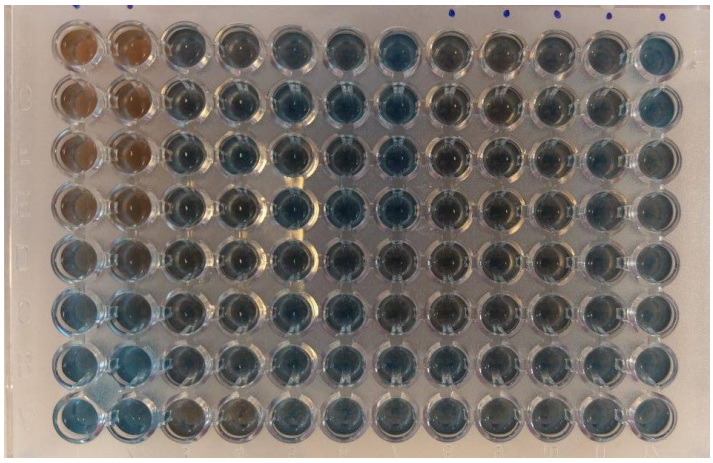

Figure 7 Bradford protein assay

Bradford protein assay for quantification and normalization of protein samples prior to SDS-PAGE and Western blotting. Serial dilutions of a known protein standard (BCA assay) were used to create a standard curve (leftmost columns). Samples for Western blotting were loaded based on their protein concentration as determined by the Bradford assay.

4.3.4 Electrophoresis and Detecting Bands

After having proper samples for loading in 8–12% SDS-PAGE, we loaded 4–8 µl of samples inside the gels to perform electrophoresis. Following the completion of the electrophoresis process, we transferred the proteins from the gels to PVDF membranes using the Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell (1,703,940). Then, by using a 5% Milk Tris-buffered saline-Tween-20 (TBST) solution, we blocked the membranes for one hour at room temperature. The incubation step of the target-specific antibody took place at night in the 4 °C fridge. The primary antibodies used for incubation include: HSL (1:1000, cell signaling 18381S), ATGL (1:1000, cell signaling 2439 S), mTOR (1:1000, cell signaling 2983S), Sirt3 (1:1000, cell signaling 2627), PCYT2 (1:1000, Thermofisher PA5-90,366), AMPK-α (1:1000, cell signaling 2532), eNOS (1:1000, abcam ab76198), Phospho-eNOS (Ser1177) (1:1000, cell signaling (9571), Sirt1 (1:1000, abcam, ab110304), LONP1 (1:3000, Proteintech − 66,043–1-Ig), SDHA (1:3000, SantaCruz—sc-98253), CS (1:1000, abcam,ab96600), PGC-1α (1:3000,

Novusbio, NBP1-04676), Fis1 (1:1000, SantaCruz, sc98900), Mfn1 (1:1000, SantaCruz, sc50330), FNDC5 (1:1000, abcam, ab174833), GPR41 (1:1000, Thermofisher, PA5-75,521), GPR43 (1:500, Thermofisher, PA5-111,780), nNOS (1:1000, BD Transduction Laboratories, 610,309), NAMPT/Visfatin (1:1000,abcam, ab45890), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:40,000;Sigma-Aldrich, 9001–50-7), α-Tubulin mouse (1:20,000, Sigma-Aldrich,T6199). On the following day, the incubated membranes went through a washing process with TBST buffer 4 times for 8 min at room temperature, then we performed the second antibody incubation process at room temperature for one hour according to the host species of primary antibody. The anti-rabbit and anti-mouse IgG HRP-conjugated secondary antibodies (Jackson Immunoresearch) were used, which were diluted 1:10,000 in a 5% milk TBST solution. Next, we washed the membranes four times for 8 minutes at room temperature using TBST buffer. The washed membranes were incubated for one minute in chemiluminescent reagent SuperSignal™ West Pico PLUS Chemiluminescent Substrate REF-34580. We used the AZURE 400 Visible Fluorescent Imager (AZI400-01) for detecting specific protein bands on the membranes by applying Wide Dynamic Range cumulative imaging mode. After obtaining band spots on the membranes, we used ImageJ software version 1.53t to quantify them. The values were normalized by housekeeping proteins, which in our study were GAPDH and α-Tubulin. Also, the phosphorylation ratio was calculated by using the same PVDF membrane after stripping the phosphorylated form of the proteins and incubating the same membrane in the total protein antibodies.

4.4 Statistical analysis

We used GraphPad Prism version 9.1.0 software to perform statistical analysis and analyze experimental data. Our reported data includes mean values with either a standard deviation (SD) or a standard error of the mean (SEM). The significance level for all analyses was set at $p<0.05$.

Since our experimental design comprised four distinct groups, we implemented a two-way analysis of variance (ANOVA) as the primary statistical method. This technique

allowed us to assess the significance of differences among groups and experimental contexts.

We continued our analysis by applying Fisher's LSD post hoc test to detect significant differences among different groups of the study. We selected this post hoc test due to its effectiveness in identifying comparisons, which enables a thorough exploration of the intricate variances in our experimental data. These statistical methods strengthened the validity of our data analysis and made our statistical results more significant and reliable.

5. Results

5.1 The effects of PGC-1α overexpression on running distance

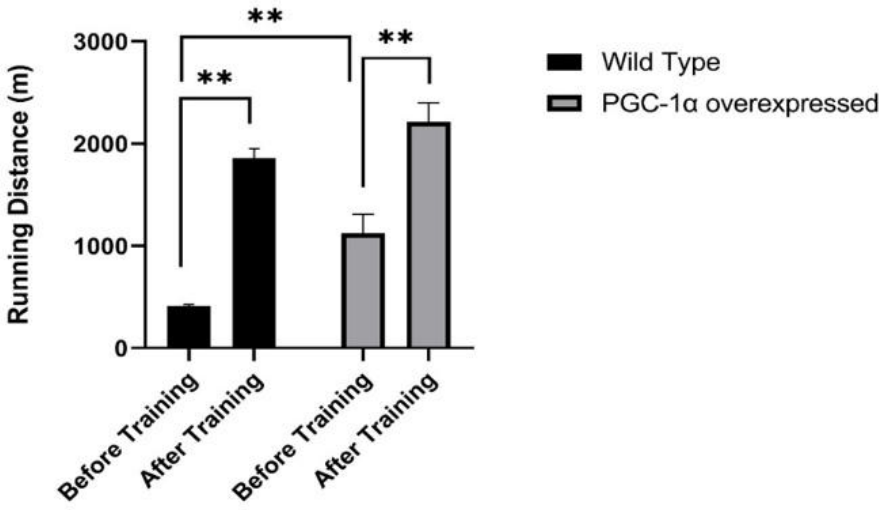

Figure 8 Comparison of average running distance to exhaustion between wild type and transgenic exercise groups before and after exercise training between the wt-Ex and PGC-1α-Ex groups. Results are expressed as mean ± SEM, ** $p < 0.01$.

The comparison of running distances to exhaustion showed a statistically significant difference between the Wild Type (wt-Ex) and PGC-1α overexpressed (PGC-1α-Ex) groups, both at baseline and after exercise training (Figure 8). This important finding suggests that overexpressing PGC-1α in skeletal muscle improves endurance ability and probably increases resistance to exhaustion during exercise. Significantly, even before exercise training, PGC-1α overexpression was associated with longer running distances, highlighting its innate function in controlling muscle performance. These findings suggest that PGC-1α may facilitate modifications such as higher mitochondrial content and increased fatty acid oxidation capacity, that help delay fatigue during endurance exercise. The advantages of these findings might be important in developing strategies for preventing muscle fatigue, improving endurance performance, or controlling muscle weakness in different diseases related to muscle function.

5.2 The effects of PGC-1α overexpression and exercise training on mitochondrial and oxidative stress marker levels

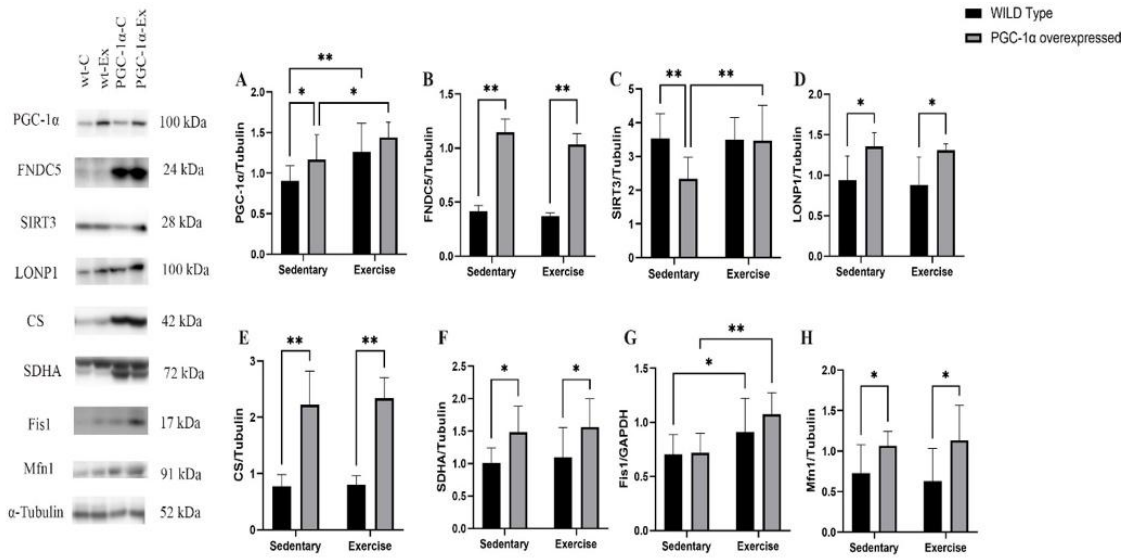

Figure 9 Oxidative stress marker levels and proteins in the mice quadriceps muscle The effects of PGC-1α overexpression and exercise training on mitochondrial and oxidative stress marker levels and proteins in the mice quadriceps muscle. Sedentary (n = 11) groups and exercise groups (n = 9). Results are normalized to GAPDH and α-Tubulin and expressed as mean ± SE, * $p < 0.05$, ** $p < 0.01$.

Comparing wild-type mice with those overexpressing PGC-1α, western blot analysis showed overexpression of PGC-1α in mice's skeletal muscle resulted in significant differences in protein expression levels linked to their mitochondrial function. The experiment's results clearly showed significant differences in the protein levels of the two groups. Increased PGC-1α (A), FNDC5 (B), LONP1 (D), CS (E), SDHA (F), and Mfn1 (G) levels were linked to PGC-1α overexpression, but SIRT3 (C) levels were lower. According to these results, PGC-1α overexpression may have an impact on several metabolic pathways, such as those involved in mitochondrial biogenesis (CS, SDHA), mitochondrial dynamics (Mfn1), and possibly cellular signaling (FNDC5, SIRT3). Most importantly, the effects of exercise training on protein levels vary based on whether PGC-1α overexpression was applied or not. The exercise training regimen increased the Wt-Ex group's PGC-1α (A) and Fis1 (G), the proteins that play the main role in the

mitochondrial fission process. On the other hand, PGC-1α (A), SIRT3 (C), and Fis1 (G) all showed increases in response to exercise training in the PGC-1α-Ex group. These findings demonstrate the intricate relationship between skeletal muscle changes brought on by exercise and PGC-1α overexpression (Figure 9).

5.3 The effects of PGC-1α overexpression and exercise training on metabolic and adaptive capacity-related markers

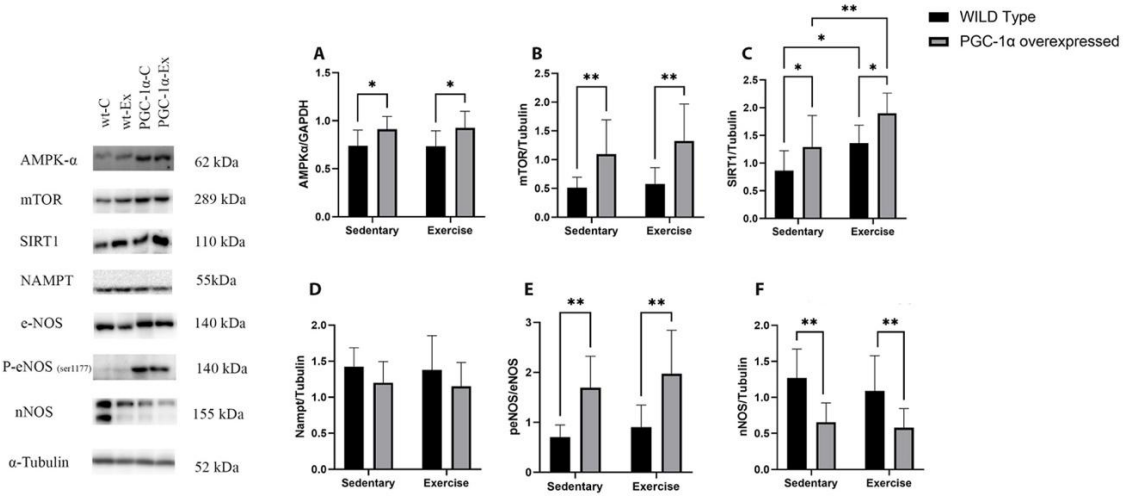

Figure 10. The effects of PGC-1α overexpression and exercise training on metabolic and adaptive capacity-related protein levels in the mice quadriceps muscle. In comparison to wild-type mice, PGC-1α overexpressed mice showed a significant increase in AMPK-α (A), mTOR (B), SIRT1 (C), and peNOS/eNOS (E), whereas a significant decrease has been observed only in nNOS (F). Also, SIRT1 (C) protein levels increased in both trained groups (wt-Ex and PGC-1-Ex), where the increase was more pronounced among the PGC-1-Ex group. Sedentary (n=11) groups, Exercise groups (n=9). Results are normalized to GAPDH and α-Tubulin and expressed as mean ± SE, * $p < 0.05$, ** $p < 0.01$.

Comparing wild-type mice with those overexpressing PGC-1α, western blot analysis showed overexpression of PGC-1α in mice skeletal muscle resulted in higher levels of AMPK-a, a key marker for cellular energy (Figure 10A). Furthermore, as seen in Figure 10B, these animals had elevated levels of mTOR, a protein synthesis and cell proliferation

factor, indicating a possible connection between PGC-1α and mTOR signaling pathways. Furthermore, as shown in Figure 10C, the PGC-1α overexpressed animals showed elevated levels of SIRT1 protein, a crucial regulator of cellular processes, highlighting the complex interactions between these regulatory elements. The endothelial phosphorylated NOS (peNOS) to eNOS ratio significantly increased in transgenic animals (Figure 10E), suggesting a possible effect of PGC-1α on the vascularization of the skeletal muscle. On the other hand, animals that overexpressed PGC-1α showed decreased levels of neuronal NOS (nNOS) (Figure 10F), suggesting that PGC-1α may have different regulatory functions in controlling subtypes of NOS in skeletal muscle. Moreover, analyzing the effects of exercise training showed that both the PGC1-Ex and wt-Ex groups significantly increased SIRT1 protein content (Figure 10C). This finding contributes to the possibility of a synergistic effect between exercise and PGC-1α, which could account for the observed improvements in metabolic flexibility and muscle performance.

5.4 The effects of PGC-1α overexpression and exercise training on lipid metabolism markers

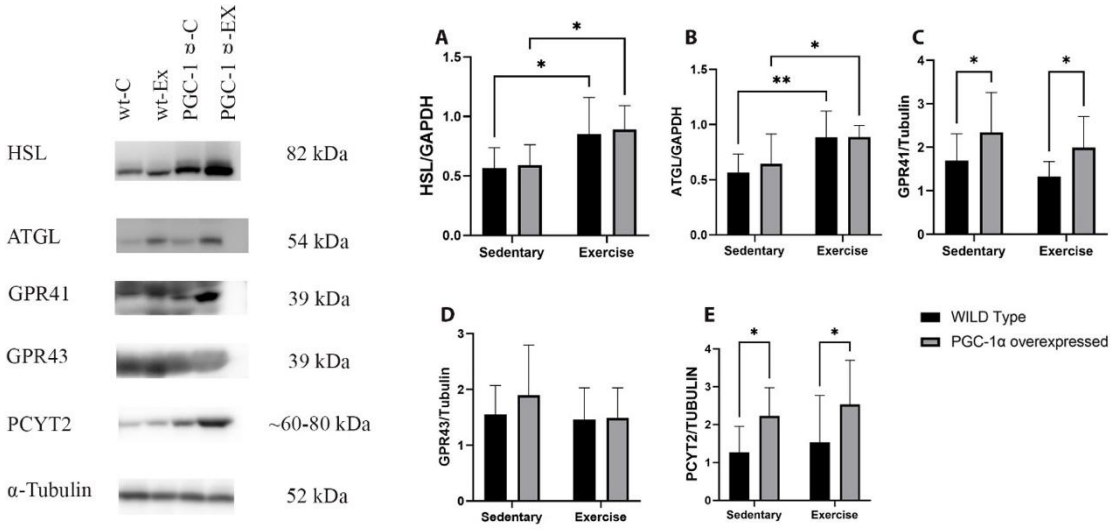

Figure 11. The effects of PGC-1α overexpression and exercise training on the levels of lipid metabolism related proteins in the mice quadriceps muscle. Lipid metabolism-related proteins in the mice quadriceps muscle PGC-1α overexpressed mice showed a significant increase in GPR41 (D) and PCYT2 (E) when compared to wild-type animals. Additionally, exercise training significantly increased the levels of HSL (A) and ATGL (B) key lipases involved in triglyceride breakdown among both groups (wt-Ex and PGC-1α-Ex). Sedentary (n = 11) groups and exercise groups (n = 9). Results are normalized to GAPDH and α-Tubulin and expressed as mean ± SE, * $p < 0.05$, ** $p < 0.01$.

Comparing wild-type mice with those overexpressing PGC-1α, western blot analysis showed that overexpression of PGC-1α in mice skeletal muscle resulted in higher levels of GPR41 (C) and PCYT2 (E), suggesting that PGC-1α may have regulatory functions in the expression of these genes. In the case of the effects of exercise training on lipase levels, the case of the effects of physical training, this intervention increased the levels of HSL (A) and ATGL (B) key lipases involved in triglyceride breakdown among both groups (wt-Ex and PGC-1α-Ex). (Figure 11).

6. Discussion

In the present research, we investigated the role of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha, a key regulator of mitochondrial biogenesis and cellular energy metabolism, together with endurance training and their combined effects on intermuscular lipid metabolism, mitochondrial function, and metabolic adaptation in skeletal muscle. By using transgenic mice with muscle-specific PGC-1α overexpression, we aimed to evaluate whether the observed changes in lipid metabolism were mainly attributed to PGC-1α or significantly influenced by other exercise-related factors. We found that overexpressing PGC-1α significantly increased the endurance ability of mice, as shown by better performance in the standard fatigue test. Along with this, mitochondrial dynamics, mitochondrial density, energy-producing enzymes in the TCA cycle, and proteins involved in energy-production pathways were upregulated. Furthermore, our endurance exercise training intervention increased the impact of these effects and resulted in the upregulation of key lipolytic enzymes ATGL and HSL in our study, independent from PGC-1α.

6.1 PGC-1α Overexpression and Endurance Performance

Our results showed that transgenic mice with skeletal muscle-specific overexpression of PGC-1α in the exercise group had significantly better endurance performance compared to the wild-type exercise group, according to longer running distances and better resistance to fatigue in both the pre-and post-tests. This aligns with previous studies using different methods to evaluate PGC-1α effects: whole-body overexpression (J. Lin et al., 2002) and skeletal muscle-specific overexpression. Both methods of overexpression resulted in increased mitochondrial content, improved exercise performance, and a shift to slow-twitch fibers, emphasizing the positive impact of PGC-1α on skeletal muscle function and metabolism regardless of the overexpression method (Karlsson et al., 2021; Hatazawa et al., 2015b; Tadaishi et al., 2011a). We also showed that performing 10 weeks of endurance exercise resulted in improved running distance according to our standard fatigue test. These results align with previous research highlighting the role of PGC-1α in the mitochondria (Besseiche et al., 2015; Liang & Ward, 2006b; Vega et al., 2015b).

6.2 PGC-1α, Exercise, and Mitochondrial Adaptations

6.2.1 Mitochondrial Biogenesis and Function

Our biochemical analyses showed a significant increase in PGC-1α protein levels in skeletal muscle following both PGC-1α overexpression and 10 weeks of endurance exercise training. This confirms the effectiveness of our interventions and aligns with previous studies (Hatazawa et al., 2015c; Liang et al., 2009).

Our data demonstrated no changes in FNDC5 levels in response to exercise; however, their levels were significantly increased in response to overexpression. This finding is consistent with the findings of Pang et al. (2018), who found that while exercise significantly increases PGC1α expression and mitochondrial biogenesis, FNDC5 protein levels do not elevate at the same rate, suggesting that the two processes are regulated separately (Pang et al., 2018). Another research supports our results by showing that PGC-1α overexpression enhances lipolysis by upregulating the pentose phosphate pathway and irisin/FNDC5 signaling, as we observed in our study; just the levels of FNDC5 increased in response to overexpression of PGC-1α (Xiong et al., 2015). However, our results contradict the findings of the study by Tadaishi et al., which showed that overexpressing PGC1α significantly improves mitochondrial function and exercise capacity and indirectly links the upregulation of FNDC5 as a downstream effect, supporting metabolic adaptations through irisin signaling and energy regulation (Tadaishi et al., 2011b).

Previous studies have shown that PGC-1α controls mitochondrial biogenesis and has a direct effect on the amount, activity, and expression of citrate synthase in different tissues (Austin & St-Pierre, 2012; Sumi et al., 2022). Our results showing elevated CS expression in the skeletal muscle of both PGC-1α-overexpressing groups are in line with these findings. This increase suggests that overexpressing PGC-1α, even uniquely in skeletal muscle, improves mitochondrial function, as CS is a critical enzyme in the TCA cycle, reflecting denser mitochondria and improved organelle functionality (Yubero et al., 2016). The previous study by Fernandes et al. (2020) provides further support for the enhanced endurance function of the animals that was seen in our fatigue tests. The results

of our fatigue running test showed that overexpressing PGC-1α in mice improved endurance performance by enabling the animals to run farther until they were tired, which can be proven by the link between CS and PGC-1α (Fernandes et al., 2020).

Another mitochondrial energy production marker that we measured in our study, SDHA, was shown to be increased by overexpressing PGC-1α in both sedentary and exercised mice. This is in line with the widely accepted role of PGC-1α as a key regulator of mitochondrial biogenesis and function (S. Kong et al., 2022a). The observed increase in SDHA, an essential part of the SDH complex that generates energy through the TCA cycle and ETC (Nazar et al., 2019; Rutter et al., 2010), suggests that the mitochondrial respiratory capacity could have been increased in response to the overexpression of PGC-1α. Our results are consistent with previous studies showing that exercise can improve the expression of SDHA (Hwang et al., 2022; Ballarò et al., 2019). However, our research did not show any increase in SDHA expression with exercise, both in wild-type and overexpressed PGC-1α mice. It was shown that higher levels of SDHA improve the function and flexibility of mitochondria, highlighting the importance of SDH in keeping mitochondria healthy and lowering ROS levels (Burgener et al., 2019). We need more research to understand how overexpression of PGC-1α affects SDHA and how exercise-induced changes might interact with PGC-1-mediated control. Understanding these mechanisms will help to explain the complex regulation of mitochondrial function and its impact on metabolic health and exercise capacity.

6.2.2 Mitochondrial Health and Oxidative Stress

We showed a complex interaction between PGC-1α and SIRT3 in skeletal muscle, which contrasts with the findings of Kong et al. (2010), who reported that PGC-1α upregulates SIRT3 (X. Kong et al., 2010). In our study, we observed that SIRT3 levels were decreased in response to PGC-1α overexpression in skeletal muscle.

SIRT3 is a regulator of energy metabolism, including fatty acid metabolism, antioxidant defenses, and mitochondrial energy production, making this finding unexpected. However, our exercise intervention restored the decreased SIRT3 levels in the transgenic mice to normal levels. On the other hand, exercise training did not increase the levels of

SIRT3 in wild-type animals. Our findings suggest that exercise training can reduce the negative effects of PGC-1α overexpression on SIRT3 levels. These findings challenge the previously established link between PGC-1α and SIRT3 and highlight the need for further research to explain the complex regulatory mechanisms governing their interaction in skeletal muscle.

Based on our observation of LONP1 protein levels, PGC-1α overexpression resulted in a significant increase in this protein, emphasizing its function in improving mitochondrial quality control. This result is in line with the findings of Guo et al. (2022), who demonstrated the important role that LONP1 plays in regulating the quality of mitochondrial proteins by removing damaged or dysfunctional ones (Guo et al., 2022). As a potential therapeutic target for diseases involving mitochondrial dysfunction, the increased LONP1 found in our study shows this protein is essential in preventing mitochondrial failure.

6.2.3 Mitochondrial Dynamics

We examined the effects of PGC-1α overexpression and exercise on mitochondrial dynamics. In both the exercise and sedentary groups, Mfn1 levels were found to be higher in mice with overexpressed PGC-1α. This is in line with previous findings (Yu et al., 2021; Martin et al., 2014). Mfn1 is one of the key proteins that regulate mitochondrial fusion and is crucial in maintaining mitochondria's structural integrity, functionality, and quality control (Moon & Jun 2020). However, our findings differ from those of Campos et al., who reported that a 12-week exercise intervention led to increased mitochondrial fusion markers, including MFN1, as evidenced by physiological parameter evaluations and biochemical analysis of cardiac structure, autophagy, mitochondrial dynamics, and function (Campos et al., 2017).

We analyzed Fis1, a key protein involved in mitochondrial fission, which plays a crucial role in mitochondrial dynamics by breaking down mitochondria into new units. Our results showed elevated Fis1 levels following exercise, consistent with earlier studies highlighting its essential role in maintaining mitochondrial integrity and functionality (Ihenacho et al., 2021a). According to earlier reports, exercise-induced Fis1 increases

appear to be an adaptation mechanism to enhance mitochondrial energy response to training (Ihenacho et al., 2021a; Yu et al., 2020).

6.3 PGC-1α, Exercise, and Lipid Metabolism

6.3.1 Lipolytic Enzymes

Our results show that PGC-1α overexpression and exercise training affect lipid metabolism markers differently in skeletal muscle. Expressions of lipolytic enzymes, specifically hormone-sensitive lipase and adipose triglyceride lipase in our study, showed significant increases in response to endurance exercise. Our results align with earlier research that found ATGL was upregulated in response to 9- and 8-week treadmill running in rodents (Ogasawara et al., 2012; Turnbull et al., 2016). Similarly, our findings for HSL reflect the results of Liu et al. (2020), who showed that high-intensity interval training increased HSL levels and activation. These observations support the consistent role of these lipolytic enzymes in exercise-induced adaptations, suggesting that endurance training effectively improves lipid mobilization pathways.

6.3.2 Lipid Biosynthesis and PCYT2

Our study showed a new role of PGC-1α in regulating PCYT2, a key enzyme in the Kennedy pathway, which is crucial for lipid biosynthesis. Independent of exercise interventions, PGC-1α overexpression significantly increased PCYT2 levels, highlighting its multifaceted functions beyond mitochondrial biogenesis and energy homeostasis. This finding aligns with recent research underscoring PCYT2's role in maintaining muscle integrity and mitochondrial functionality (Cikes et al., 2023). As a central enzyme in phosphatidylethanolamine and lipid synthesis, PCYT2 supports cellular membrane dynamics and mitochondrial structure (Cikes et al., 2023; Y. Gao & Tian, 2023; Z. Gao et al., 2009). By upregulating PCYT2, PGC-1α may enhance lipid synthesis, improving mitochondrial efficiency and muscle health.

6.3.3 Short Chain Fatty Acid Receptors

Short-chain fatty acid receptors GPR41 and GPR43, which specialize in uptaking short-chain fatty acids produced through the fermentation of non-digestible dietary fibers by gut bacteria, are crucial components in linking gut microbiota and host metabolism (Frampton et al., 2020). Here, we show that the GPR41 receptor is detectable in our animal model, aligning with the results of the Chinese group in 2014 (G. Li et al., 2014). We observed that PGC-1α overexpression significantly increased GPR41 levels compared to wild-type animals. This improvement suggests a potential mechanism by which PGC-1α facilitates the uptake of SCFAs into skeletal muscle, which may contribute to improved energy metabolism in PGC-1α-overexpressing animals under both sedentary and exercise conditions (Mozaffaritabar et al., 2024). These findings highlight GPR41's role in mediating the metabolic benefits associated with PGC-1α overexpression.

However, our interventions failed to significantly affect GPR43 expression, suggesting its regulation may be independent of PGC-1α or responsive to other factors. This contradiction suggests that, while GPR41 and GPR43 share functional roles in SCFA metabolism, their regulatory mechanisms and physiological implications might differ. Further investigations are necessary to clarify the specific pathways through which PGC-1α influences GPR41 and whether GPR43 plays a complementary or distinct role in skeletal muscle metabolism.ß

6.4 PGC-1α, Exercise, and Signaling Pathways

6.4.1 AMPK-α and SIRT1 Signaling

In our study, we observed a significant increase in AMPK-α levels in transgenic animals overexpressing PGC-1α, consistent with the established role of PGC-1α in enhancing AMPK-α activity and promoting fatty acid oxidation. This result is supported by previous studies showing that PGC-1α functions as a coactivator for transcription factors involved in mitochondrial biogenesis and metabolic regulation, including AMPK-α. Specifically, AMPK-α plays a critical role during energy stress by regulating genes associated with fatty acid metabolism (Zuo et al., 2023; Cantó & Auwerx, 2009; J. Kim et al., 2016).

However, exercise training did not increase the level of AMPK-α in wild-type animals, contradicting the findings of the previous study (C.-C. Huang et al., 2016). Additionally, exercise did not further increase AMPK-α levels in transgenic animals, which was unexpected and might be due to differences in species, exercise protocols, or the specific muscle fiber types analyzed.

We observed upregulation of SIRT1 protein levels in the sedentary transgenic group compared to their wild-type counterparts. This result shows that PGC-1α overexpression increases SIRT1 protein levels independently of exercise. Exercise training further increased SIRT1 levels in both wild-type and transgenic groups, with transgenic animals exhibiting a greater increase compared to wild-type animals. The results on how exercise raises SIRT1 levels are like those from studies done on animals, especially the one by Koltai et al. (2010), which showed how important SIRT1 is for adapting to exercise training (Koltai et al., 2010).

6.4.2 NOS Enzymes

The higher peNOS/eNOS ratio in mice overexpressing PGC-1α reflects that more endothelial NOS (eNOS) is being activated. This is in line with PGC-1α's well-documented role in improving nitric oxide production and endothelial function (Craige et al., 2016). The PGC-1α coactivates transcription factors like nuclear respiratory factor 1, peroxisome proliferator-activated receptor gamma, and estrogen-related receptor alpha. These factors bind to the eNOS gene promoter and make it more active. Additionally, PGC-1α stabilizes eNOS mRNA, reducing its degradation and prolonging its half-life, thereby increasing eNOS protein levels (Oliveira-Paula et al., 2016; Puigserver et al., 1998). By reducing reactive oxygen species through the upregulation of antioxidant enzymes, PGC-1α also helps preserve eNOS stability and activity. These mechanisms collectively explain how PGC-1α overexpression enhances eNOS levels, leading to increase NO production and improved endothelial function. In contrast, our findings showed a reduction in neuronal NOS levels in PGC-1α-overexpressing animals. This was unexpected based on our own findings. Although AMPK-α levels were higher in our study, the higher amount did not seem to affect nNOS. These results are different from

those reported by Huber-Abel et al. (2012), who showed increased nNOS levels following exercise intervention in human skeletal muscle tissue (Huber-Abel et al., 2012). Different experimental models, exercise protocols, or the specific muscle fiber types used for the study could explain the observed differences.

6.4.3 NAMPT and NAD^+ Metabolism

Our findings indicate that NAMPT expression levels were unaffected by either exercise training or PGC-1α overexpression, which is unexpected given NAMPT's crucial role in NAD+ generation and metabolic health. Previous studies, such as those by Dahl et al., 2012; Garten et al. 2011, 2015 (Dahl et al., 2012; Garten et al., 2011, 2015). Also, the contrasting results compared to the previous study by Koltai et al. (2010), who reported elevated NAMPT expression following exercise training. Our exercise training intervention did not affect the expression of NAMPT. Overexpression of PGC-1α may change other pathways involved in NAD+ metabolism, which would counteract the long-lasting effects of NAMPT expression (Koltai et al., 2010). These observations suggest that PGC-1α may influence the NAMPT-NAD+-SIRT pathway through alternative regulatory mechanisms. Future research should explore this pathway in more detail, focusing on whether sirtuin activity and NAD+ levels change independently of NAMPT expression, this illustrates the adaptive processes involved in the growth of skeletal muscle and its impact on metabolic health.

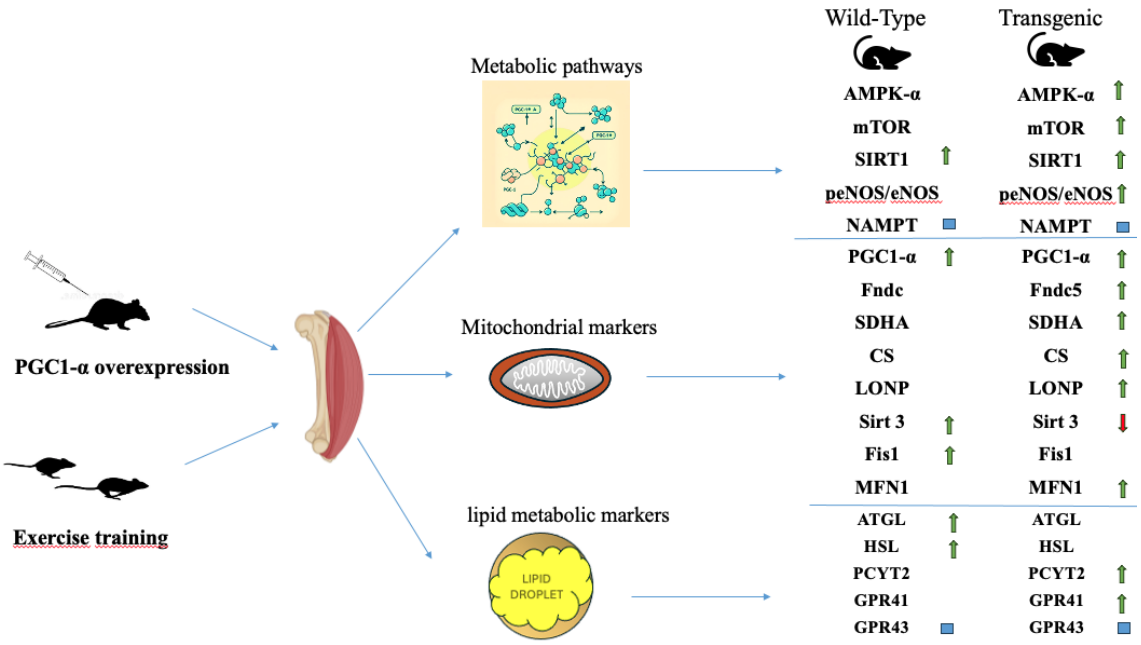

Figure 12 Summary of Study Findings: Effects of PGC1-α Overexpression and Exercise Training on Skeletal Muscle Metabolism

The figure illustrates the study's key findings, comparing wild-type (left) and PGC-1α transgenic (right) mice. It presents a biochemical analysis of protein expression after 10 weeks of endurance training or sedentary conditions, focusing on mitochondrial density, function, and dynamics markers, lipid metabolism-related indicators, and adaptive metabolic signaling pathway markers. Adaptations are represented with arrows indicating upregulation (↑), downregulation (↓), or no change (■).

7. Conclusion

In conclusion, our findings provide strong evidence that PGC-1α plays a key role in skeletal muscle adaptation to exercise by modulating mitochondrial dynamics and key signaling pathways. Additionally, PGC-1α and exercise independently influence lipid breakdown. The novel findings regarding the interaction between PGC-1α and GPR41, PCYT2 (Figure 12) are important, suggesting potential new pathways for therapeutic intervention.

In this section, we conclude that our hypothesis evaluation supports the role of PGC-1α in modulating mitochondrial dynamics and lipid metabolism while also highlighting the independent effects of exercise on these processes.

Hypothesis 1: PGC-1α overexpression in skeletal muscle and endurance exercise training improve mitochondrial health and function markers, leading to enhanced endurance performance.

Accepted: Our results validate our first hypothesis. This is shown by PGC-1α overexpressing mice which can run longer distances and are less likely to get tired, which is in line with PGC-1α's known role in supporting mitochondrial function and oxidative metabolism. The results also indicate that exercise training positively affected performance, as evidenced by post-test assessments and an increase in PGC-1α levels in wild-type mice that underwent the exercise training program. Additionally, higher levels of important mitochondrial markers like CS, SDHA, LONP1, and PGC-1α, which indicate a greater capacity for mitochondrial biogenesis, health, and energy production marker adaptation. This further supports our hypothesis that both PGC-1α overexpression and exercise training enhance endurance performance.

Hypothesis 2: PGC-1α overexpression in skeletal muscle and endurance exercise training will improve triglyceride breakdown markers, leading to increased reliance on fat as an energy source in the quadriceps muscle tissue of mice.

Partially accepted. The hypothesis that exercise training increases triglyceride breakdown enzymes is confirmed, as exercise training led to an increase in lipolytic enzymes. In our study, overexpression of PGC-1α alone did not significantly affect the enzymes HSL and ATGL. However, animals with PGC-1α overexpression that underwent exercise showed higher enzyme levels than those exercising wild-type animals. Furthermore, to understand the broader metabolic effects related to triglyceride breakdown, we measured the expressions of the fatty acid receptors GPR41 and GPR43 in the skeletal muscles of mice. Previous research has shown that these receptors affect the activation of AMPK-α, an enzyme that increases PGC-1α activity resulting in elevated number and improved function of mitochondria (figure 4). Our findings indicated that GPR41 expression increased in response to PGC-1α overexpression, whereas GPR43 expression remained unchanged. Interestingly, our results showed increased expression of PCYT2, an important lipid biosynthesis regulator for muscle health and aging, which may play a role in muscle adaptation to metabolic changes induced by PGC-1α overexpression. Notably, this increase in PCYT2 expression was not observed with exercise alone. This adds a new dimension to understanding how lipid metabolism is regulated in skeletal muscle.

Hypothesis 3: PGC-1α overexpression in skeletal muscle and endurance exercise training modulate the expression of key enzymes involved in mitochondrial dynamics, contributing to improved metabolic flexibility and exercise adaptation in the quadriceps muscle tissue of mice.

Accepted: Our results confirm this hypothesis, showing that PGC-1α overexpression increases Mfn1 levels, enhancing improved structural integrity and fused mitochondria, while endurance exercise significantly increases Fis1 levels, a marker of the process of breaking down into new mitochondria. The findings from the PGC-1α-Ex group suggest that PGC-1α and exercise are linked in regulating mitochondrial dynamics, validating our third hypothesis that both interventions influence the proteins involved in this process.

Hypothesis 4: PGC-1α overexpression in skeletal muscle and endurance exercise training change key signaling pathways, such as the AMPK-α and sirtuin pathways, in the quadriceps muscle tissue of mice.

Partially Acceptable: Our results showed that endurance training alone did not affect SIRT3 levels in wild-type mice. However, in PGC-1α overexpressing animals, endurance training effectively normalized the previously reduced SIRT3. This indicates that the effect of exercise on SIRT3 is likely dependent on the presence of PGC-1α, as evidenced by the normalization of SIRT3 levels in PGC-1α overexpressing animals. In contrast, SIRT1 levels increased in response to both interventions. PGC-1α binds to the promoter region of the SIRT1 gene and activates its transcription, thereby increasing the production of the SIRT1 protein. This interaction between PGC-1α and SIRT1 forms a positive feedback loop that regulates cellular energy homeostasis and mitochondrial function. However, regarding AMPK-α as an upstream regulator of PGC-1α, we found that PGC-1α can coactivate AMPK-α, leading to increased AMPK-α activity. PGC-1α can reduce the production of reactive oxygen species and upregulate antioxidant enzymes. This is supported by our results, which show increased phosphorylation of the eNOS enzyme. Thus, we conclude that PGC-1α helps to preserve the eNOS protein and maintain its activity. Unexpectedly, nNOS levels decreased in response to PGC-1α overexpression and remained unchanged by exercise. These findings partially support our hypothesis that PGC-1α overexpression and endurance exercise have distinct and independent effects on key signaling pathways. However, the combined effects of PGC-1α overexpression and endurance exercise did not consistently produce synergistic outcomes, highlighting the complex nature of their interactions as observed in the study. To better understand how PGC-1α and exercise training affect skeletal muscle adaptation, more research is required to explore the specific mechanisms.

8. Summary

The role of Peroxisome proliferator-activated receptor-gamma coactivator alpha (PGC-1α) in fat metabolism is not well known. In this study, we compared the mechanisms of muscle-specific PGC-1α overexpression and exercise-related adaptation-dependent fat metabolism. PGC-1α trained (PGC-1α Ex) and wild-trained (wt-ex) mice were trained for 10 weeks, five times a week at 30 min per day with 60 percent of their maximal running capacity. The PGC-1α overexpressed animals exhibited higher levels of Fibronectin type III domain-containing protein 5 (FNDC5), 5' adenosine monophosphate-activated protein kinase alpha (AMPK-α), the mammalian target of rapamycin (mTOR), Sirtuin 1 (SIRT1), Lon protease homolog 1 (LONP1), citrate synthase (CS), succinate dehydrogenase complex flavoprotein subunit A (SDHA), Mitofusin-1 (Mfn1), endothelial nitric oxide synthase (eNOS), Hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), G protein-coupled receptor 41 (GPR41), and Phosphatidylcholine Cytidylyltransferase 2 (PCYT2), and lower levels of Sirtuin 3 (SIRT3) compared to wild-type animals. Interestingly, our findings reveal that GPR41 and PCYT2 levels were particularly affected by PGC-1α overexpression, highlighting their novel roles in lipid metabolism and cellular adaptation to enhanced mitochondrial activity. Exercise training increased SIRT1, HSL, and ATGL levels in both wild-type trained (WT-Ex) and PGC-1α trained (PGC-1α Ex) groups. PGC-1α has a complex role in cellular signaling, including the upregulation of lipid metabolism-associated proteins. Our data reveals that although exercise training mimics the effects of PGC-1α overexpression, it incorporates some PGC-1α-independent adaptive mechanisms that alter signaling pathways involved in fat uptake and regulation of lipid metabolism.

Keywords: PGC-1α, mitochondrial biogenesis Fat metabolism, Exercise-related adaptation, Cell Signaling

Összefoglalás

A Peroxisoma proliferátor-aktivált receptor-gamma koaktivátor alfa (PGC-1α) szerepe a zsíranyagcserében még nem teljesen ismert. Ebben a tanulmányban összehasonlítottuk az izom-specifikus PGC-1α túlexpresszió és az edzéshez kapcsolódó adaptáció-függő zsíranyagcsere mechanizmusait. PGC-1α edzett (PGC-1α Ex) és vad típusú (wt-ex) egereket 10 héten keresztül, heti öt alkalommal, napi 30 percig edzettünk a maximális futási kapacitásuk 60 százalékán. A PGC-1α túlexpresszált állatoknál magasabb szintű Fibronectin III típusú domént tartalmazó fehérje (FNDC5), 5' adenozin-monofoszfát-aktivált protein kináz alfa (AMPK-α), a rapamicin emlős célt fehérje (mTOR), Sirtuin 1 (SIRT1), Lon proteáz homológ 1 (LONP1), citrát-szintáz (CS), szukcinát-dehidrogenáz komplex flavoprotein alegység A (SDHA), Mitofuzin-1 (Mfn1), endothelialis nitrogén-monoxid szintáz (eNOS), hormonérzékeny lipáz (HSL), adipóz-triglicerid-lipáz (ATGL), G-fehérje-kapcsolt receptor 41 (GPR41) és foszfatidilkolin-citidililtranszferáz 2 (PCYT2) szintet, valamint alacsonyabb Sirtuin 3 (SIRT3) szintet figyeltünk meg a vad típusú állatokhoz képest. Érdekes módon megállapításaink szerint a GPR41 és a PCYT2 szintje különösen érzékeny volt a PGC-1α túlexpresszióra, ami kiemeli ezek újszerű szerepét a lipidanyagcserében és a fokozott mitokondriális aktivitáshoz való sejt szintű alkalmazkodásban. Az edzés megnövelte a SIRT1, HSL és ATGL szinteket mind a vad típusú edzett (WT-Ex), mind a PGC-1α edzett (PGC-1α Ex) csoportban. A PGC-1α komplex szerepet tölt be a sejten belüli jelátvitelben, beleértve a lipidanyagcserével kapcsolatos fehérjék szintjének növelését. Eredményeink azt mutatják, hogy bár az edzés a PGC-1α túlexpresszió hatásait utánozza, bizonyos PGC-1α-tól független adaptív mechanizmusokat is magában foglal, amelyek megváltoztatják a zsír felvételével és a lipidanyagcsere szabályozásával kapcsolatos jelátviteli útvonalakat.

Kulcsszavak: PGC-1α, mitokondriális biogenezis, zsíranyagcsere, edzéshez kapcsolódó adaptáció, sejtes jelátvitel

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10. List of own publications

Publications related to the dissertation

Mozaffaritabar S, Koltai E, Zhou L, Bori Z, Kolonics A, Kujach S, Gu Y, Koike A, Boros A, Radák Z. PGC-1α activation boosts exercise-dependent cellular response in the skeletal muscle. J Physiol Biochem. 2024 May;80(2):329-335. doi: 10.1007/s13105-024-01006-1. Epub 2024 Jan 23. PMID: 38261146; PMCID: PMC11074013.

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Publications not related to the dissertation

Zsolt Radák , Dóra Aczél , Iván Fejes , Mozaffaritabar Soroosh , Gabor Pavlik , Zsolt Komka , László Balogh , Zsofia Babszki , Gergely Babszki , Erika Koltai , Kristen M. McGreevy , Gordevicius Juozas , Steve Horvath , Csaba Kerepesi Slowed epigenetic aging in Olympic champions compared to non-champions. Geroscience. 2024 Nov 27. doi: 10.1007/s11357-024-01440-5. Epub ahead of print. PMID: 39601999.

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