EFFECTS OF EXERCISE AND GENETICS ON SKELETAL MUSCLE LIPID METABOLISM WITH FOCUS ON THE LOCALIZATION AND ASSOCIATION OF LIPID DROPLETS, MITOCHONDRIA AND PLIN5

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ABSTRACT

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Low exercise capacity has been identified as a stronger predictor of morbidity and mortality relative to other commonly reported risk factors. Substrate metabolism, especially lipid storage, lipolysis and transportation in the skeletal muscles is strongly linked to aerobic capacity. The physical topographical properties of some myocellular particles and or organelles such mitochondria, lipid droplets (LDs) and perilipine-5 (Plin5) proteins in the skeletal muscle tissues have been implicated in the onset of most metabolic diseases and may also predict overall physical capacity. We tested the effect of innate high aerobic capacity (genetics) and acquired aerobic capacity (voluntary running) on the number, location and colocalization of the aforementioned particles in the gastrocnemius muscle cells, between the low and high capacity runner rats.

Muscle samples from the *gastrocnemius* muscle of the specially bred rat model with high aerobic capacity (HCR) (n=20) and low aerobic capacity (LCR) (n=20) were used in this study. The samples were obtained from 4 equal subgroups; high capacity runner control (HCR-C) (n=10), high capacity runner receiving exercise intervention (HCR-R) (n=10), low capacity runner control (LCR-C) (n=10) and low capacity runner receiving exercise intervention (LCR-R) (n=10). Immunocytochemistry, confocal microscopy and bioinformatics were used in data collections and analysis. A one-way between subject analysis of variance (ANOVA) show statistically significant difference in the content of mitochondria ($F(3,24) = 4.24$, $p<.0.05$) and PLIN5 particles ($F(3,24) = 3.8$, $p<.005$). There were no significant differences in the number of LD ($F(3,24) = 0.43$, $p>0.05$) or in the colocalization of the LD and PLIN5 particles ($F(3,24) = 1.2$, $p>.005$) and LD and COXIV particles ($F(3,24) = 1$, $p>.005$) across the groups.

The result suggests a strong interplay between genetic background and exercise stimulus. These factors may influence the skeletal muscle properties at the cellular level, effect of which may not only affect performance component but also health component of physical fitness. Physical activity may, however, enhance the cellular function or reverse genetic lags in cellular properties.

Keywords: Genetics, Lipid droplet, Colocalization, Physical activity, Metabolism.

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LIST OF TERMS AND ABBREVIATION

ACBPs	Acyl-CoA binding proteins
ACAT	Acyl-CoA: cholesterol acyltransferase
Acyl-CoA	Acyl-coenzyme A
ATGL	Adipose triglyceride lipase
BCAAs	Branched-chain amino acids
DGAT	Diacylglycerol acyltransferase
ER	Endoplasmic reticulum
FABPs	Fatty acid binding
FFA	Free fatty acid
IMF	Intermyofibrillar
IMTG	Intramyocellular Triglycerides
LDs	Lipid droplets
MBOAT	Membrane-bound O-acyltransferase
PLIN5	Perilipin-5
PBS-	Saponin in Phosphate Buffer Saline in Phosphate Buffer Saline
SE	Sterol esters
TG	Triglyceride

Contents

1 INTRODUCTION

There has been lots of interest in the interaction of exercise and genetics on health and physical capacity for decades (Koch & Britton, 2001). Significant efforts are geared toward the understanding of heritability of physical capacity and role of exercise in physiological composition or characteristics of varied species. A suitable and quantifiable tool for measuring exercise performance is the aerobic capacity (Karvinen et al., 2015); defined as the functional capability of the organs (heart, lungs, liver, muscles, etc.) or systems (cardiorespiratory, musculoskeletal system, etc.) of the body to effectively utilize oxygen during physical activity or exercise (Henderson et al., 2002; Wagner et al., 2002; Wang et al., 2012). Low exercise capacity was identified as stronger predictor of morbidity and mortality relative to other commonly reported risk factors such as obesity; type 2 diabetes, cardiovascular disease, (Park et al., 2016; Schwarzer et al., 2016).

Many studies from high-capacity rats (HCR) and low-capacity rats (LCR) model revealed that selection for low versus high intrinsic aerobic exercise capacity could unveil the predisposition to many differentials metabolic and cardiovascular risk factors. Exercise capacity and cardiovascular fitness have been shown to be highly predictive of metabolic health, including lower fat mass, higher insulin sensitivity, lower blood pressure and, importantly age-adjusted mortality (Overmyer et al., 2015). LCR was observed to develop cardiovascular and metabolic risks, including large gain in visceral adiposity, increased blood pressure, dyslipidaemia, endothelial dysfunction occurring within carotid arteries, and insulin resistance (Høydal et al., 2014; Wisløff et al., 2005). LCR, relative to HCR, harbour numerous clinically relevant conditions, including increased susceptibility to cardiac ventricular fibrillation (Lujan et al., 2006); hepatic steatosis (Morris et al., 2014; Thyfault et al., 2009); disordered sleep (Muncey et al., 2010); diminished behavioural strategies for coping with stress (Burghardt et al., 2011); increased sensitivity to the deleterious effects of a high-fat diet (Morris et al., 2014; Noland et al., 2007); and reduced capacity for oxidation of lipids in skeletal muscle (Lessard et al., 2009; Rivas et al., 2011), liver (Szary et al., 2015; Thyfault et al., 2009), and heart (Wisløff et al., 2005). The segregation of low intrinsic endurance exercise capacity with low health status provided unbiased evidence in support of the notion that dysfunctional capacity for energy metabolism, a term recently referred to as ’energy transfer hypothesis (ETH)′ may be fundamental to the root cause of common morbidity or altered functionality of an

individual. Although a direct cause-effect relationship has not been proven, observations support the notion that impaired regulation of oxidative pathways in mitochondria may be a common factor linking reduced total-body aerobic capacity to cardiovascular and metabolic disease (Ylikallio & Suomalainen, 2012), and numerous research efforts are elucidating this phenomenon.

The effort of this research, therefore, is to take a closer look at the morphological variations in the skeletal muscle cell of HCR and LCR rat model, with emphasis on the localisation of important agents identified to contribute to lipid metabolism. This presents a unique opportunity to identify and compare the morphological characteristics of lipid droplets (LDs), perilipin-5 (PLIN5) and mitochondria in the skeletal muscle cells of genetically and activity disparate organisms. Since it has been shown that lipid synthesis is highly compartmentalised in eukaryotic cells, regulated contacts between LDs and other organelles are therefore likely to be critical for cellular homoeostasis (Barbosa et al., 2015). More importantly, the knowledge of the structural interaction may provide useful information that could contribute to our understanding of the mechanistic processes by which identified particles interact and, or play a role in the development or amelioration of common illnesses such as type 2 diabetes, insulin resistance, obesity, cancer, hypertension, cardiovascular diseases and overall physical capability of individuals.

2 REVIEWS OF LITERATURE

2.1 Skeletal muscle lipids overview

2.1.1 The chemistry of lipid droplets

The human organism is made up of varying molecular compounds and constituents, with water 60%, proteins 17%, lipids 16% and carbohydrates 5%, being the most abundant relative to weight. These compounds invariably compose the major cells, tissues and organs of the body such as the myocytes, adipose tissue and the heart, respectively. The adipose tissue is predominantly made up of about 90% lipids while the muscle tissue is majorly composed of proteins and other compounds at varying proportion. The composition and structure of these molecules define to a considerable extent their significant roles and invariably their functions and contribution to the entire homoeostasis of the organism. The skeletal muscle tissue is a major composite of animal anatomical structure, responsible for about one-third of the total body mass. Apart from it popular functions of support and movement, it plays other numerous important roles especially in the metabolic process and maintenance of homeostasis of the entire body system.

The skeletal muscle was shown to harbour some deposit of fat, observed as neutral lipids. These fat deposits are believed to be dynamically stored in organelles named LDs. LDs in addition to glycogen store and other energy sources from circulation are opined to play essential roles in energy provision during exercise (Gacesa & Schick, 2016; Kimmel & Sztalryd, 2014; MacPherson & Peters, 2015; Wu et al., 2015). The first mention of something close to lipid droplet can be traced back to van Leeuwenhoek in 1974. However, it is fast becoming a common knowledge nowadays that all organisms use LDs as cellular storage sites for neutral lipids (Yang et al., 2012). In humans, they exist most abundantly in adipose cells and steroid-producing cells but can be found in virtually any kind of cell (Tauchi-Sato et al., 2002). LDs were for decades referred to as liposomes and subsequently as lipid bodies, fat bodies, oil bodies, spherosomes or adipocytes (Stone et al., 2009). Owing to its composition and interaction with other particles in the skeletal

muscle cells and other important organs and tissues in mammals, this unique organelle has been implicated in many cellular functions, physiological and pathological conditions (Thyfault et al. 2017). This revelation, therefore, brings to the fore, the important contribution of the skeletal muscle in the entire metabolic process, and perhaps the salient contribution of LDs to body fuel supply, especially during physical activity. However, the mechanism of LD biogenesis and catabolism remain a subject of debate.

2.1.2 LD morphology and composition

Lipid droplets are a reservoir of lipids and dynamic cellular organelles composed of a hydrophobic core of neutral lipids surrounded by phospholipid monolayer (Salo et al., 2011; Tauchi-Sato et al., 2002; Wilfling et al., 2014). A giant (up to 200 µm in diameter), unilocular LD often occupies the entire cytoplasm of white adipocytes, specialising in energy storage. In contrast, many much smaller LDs (usually less than 10 µm in diameter) are found in brown adipocytes. Small LDs are also found in normal liver cells (Cheng et al., 2014). The smallest mature intermyofibrillar (IMF) LDs are reported to range from 250-500 nm (Wang et al., 2013; Wilfling et al., 2014), while simulations predict nascent LD size of 50-100 nm in diameter (Duelund et al., 2013).

The core contains mostly triglyceride (TG) or sterol esters (SE) depending on the cell types. LDs of adipocytes contain primarily TG, and those of macrophage foam cells contain mostly SE (Wilfling et al., 2013). The surfaces are coated with specific proteins believed to contribute to the organelle breakdown and build-up. There are about 50-200 different proteins on the surface of an LD in a cell, depending on the size of the LD and composition of the proteins (Hsieh et al., 2012). Polar lipids such as sterols are found in relative quantity in various cells with phosphatidylcholine (PC) being the main surface phospholipid. Phosphatidylethanolamine (PE) and phosphatidylinositol mostly coat the mammalian LDs (Vigelsoe et al., 2015)

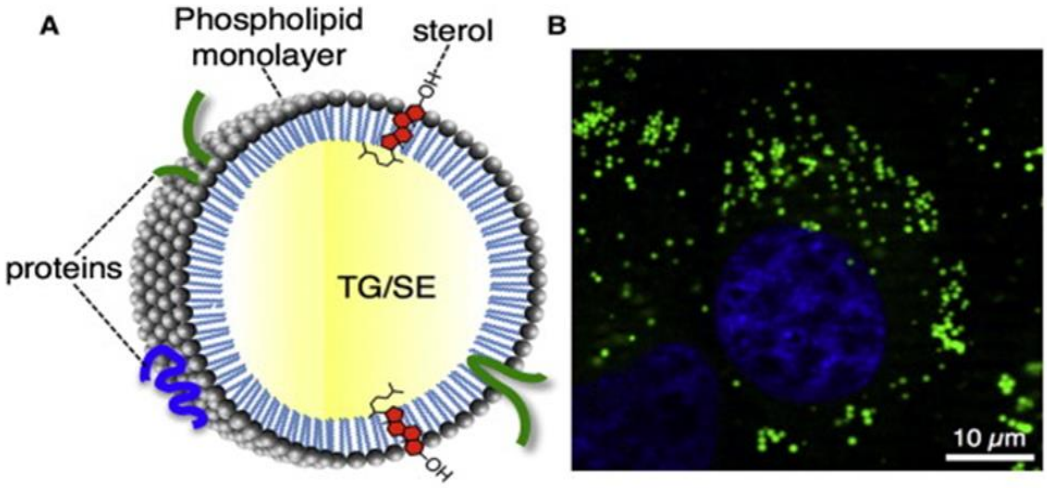

FIGURE 1. Model describing the structure of the lipid droplet. A) Showing the basic structure of LD in cartoon model with the inside made of the lipid core of Triglycerides or Sterol Ester surrounded by the phospholipid monolayer B) shows the structure of LDs in cultured cells

2.1.3 LD biogenesis and synthesis

The precise processes or molecular mechanism involved in the formation of LDs remains unclear. Several models of LD formation have, however, been proposed. Some of the models proposed include the following among others; a) endoplasmic reticulum (ER) budding, where LDs grow from ER bilayer and remain connected to it or bud off (Walther & Farese, 2009), b) disc-shaped bicelle formation in which an entire lipid lens is extracted from ER (Ploegh, 2007), c) Vesicular budding, in which a bilayer vesicle forms, followed by filling of the bilayer intramembranous space with neutral lipids (Walther & Farese, 2009) and d) the "egg-cup" model, in which an LD grows within a concave depression of the ER through transport of neutral lipids from the ER (Robenek et al., 2006). Evidence generic to most model, however, suggests that LDs could be derived either from other existing LDs by fission or formed de novo (Long et al., 2013). The latter is the most favoured in eukaryotic cells currently by available research. De novo formation involves the synthesis of LDs in the endoplasmic reticulum (ER), where the enzymes that synthesise neutral lipids are as well found (Ohsaki et al., 2014).

Yen et al. 2009 demonstrate that neutral lipid are synthesised from fatty acyl-CoAs and lipid alcohols by enzymes of the membrane-bound O-acyltransferase (MBOAT), localise usually to the ER. Meyers et al. 2016 using a yeast cell, show that TG-enriched LDs preferentially form around the nucleus, but SE-enriched LDs localise away from the nucleus tip. In mammalian cells, acyl-coenzyme A (acyl-CoA): diacylglycerol acyltransferase (DGAT) enzymes, DGAT1 and DGAT2, synthesise TG, and acyl-CoA: cholesterol acyltransferase (ACAT) enzymes, ACAT1 and ACAT2, generate SEs (Walther et al. 2012; Stone et al 2009; Kuerschner et al. 2008). The above claims appeared consistent with the hypothesis that, the LD surface is derived from the cytoplasmic leaflet of the endoplasmic reticulum membrane and may be continuous to it (Tauchi et al. 2002; King et al. 1994; Mackinnon et al. 1992; Hamilton and Small 1981). Wilfling et al. 2015 recently proposed a stepwise model of lipid droplet formation, which appear to support and consolidate the models earlier proposed. The model highlights the synthesis of neutral lipids, the formation of the lens and the birth of nascent LDs sequentially in the ER.

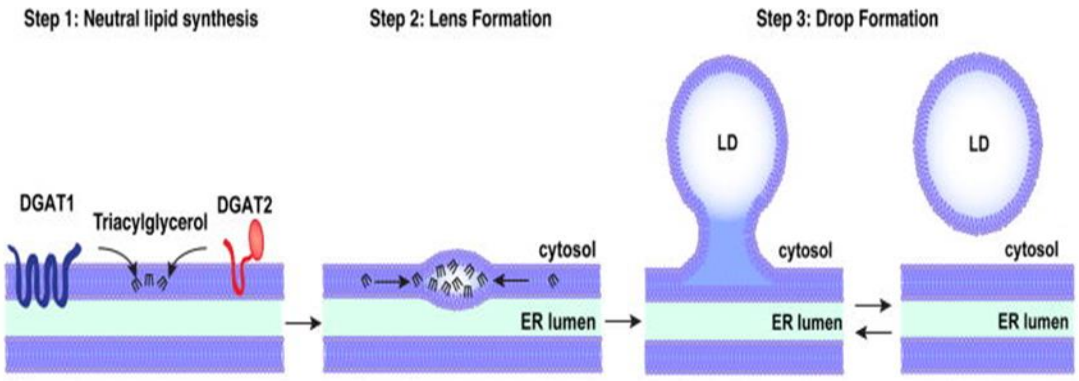

FIGURE 2. A stepwise model of lipid droplet formation involving: Lipid droplets form in at least three discrete steps; (1) Neutral lipids are synthesised in the ER and accumulate within the bilayer. Neutral lipids are highly mobile in the bilayer and may spontaneously aggregate based on thermal fluctuations and electrostatic interactions with integral membrane proteins or other lipids. (2) Once the local concentration of neutral lipid reaches a critical threshold, a lens will form as the oil phase coalesces. (3) As the lens accumulates additional neutral lipids, the bilayer deforms and a nascent lipid droplet buds into the cytoplasm, possibly via a de-wetting mechanism. The nascent droplet might remain attached to the ER or separate completely (Wilfling et al., 2014).

2.1.4 LD growth and expansion

LDs could undergo dramatic changes in response to internal or external factors, enlarging or shrinking in size, especially during muscle contraction. The magnitude of LDs in liver cells can increase during hepatic steatosis, given that cells respond to lipid overload by increasing the number and or volume of LDs (Yang et al., 2012). However, larger LDs are suggested to emerge from the relatively smaller nascent LDs either by the growth of LDs or joining of two LDs to form a single, larger LD (Wilfling, et al., 2014). Also, the joining of two LDs to form a larger LD can occur either by slow/atypical fusion or ripen in a rapid/homotypic fusion as observed commonly in adipocytes and mutant cells (Thiam et al., 2013; Yang et al., 2012).

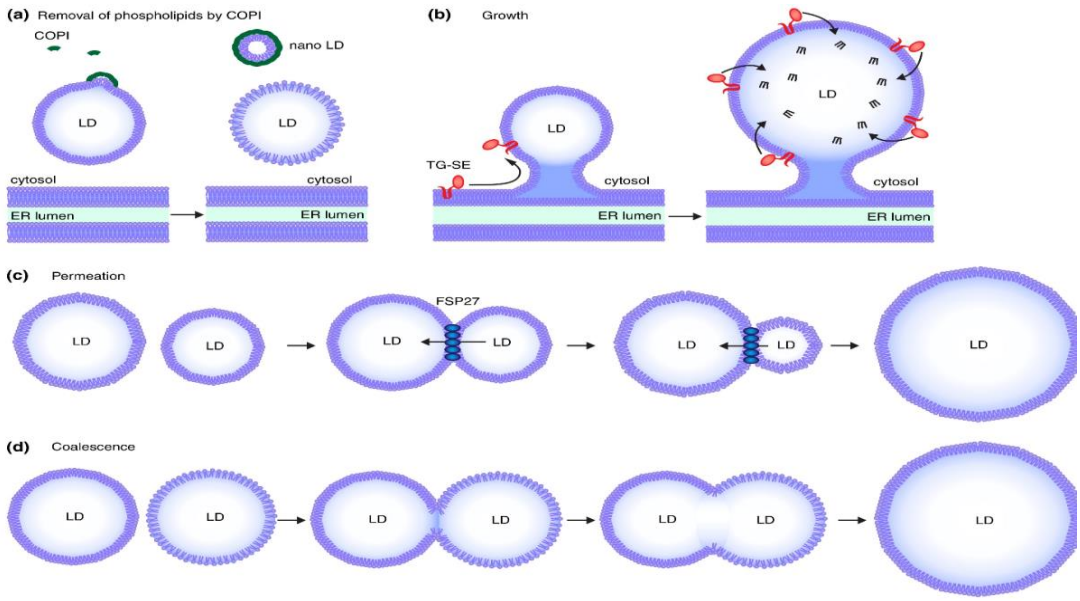

FIGURE 3. The process of LD growth involves two major mechanism embodied in series of reactions (a) The COPI machinery buds small Nano-LDs from a mature LD leading to a reduction of phospholipids on the LD surface. This leads to an increase in surface tension facilitating interactions of the LD with the ER. (b) Once connections are established, a subset of TG synthesis enzymes can re-localize to the LD surface to locally produce TG, which, in turn, leads to the growth of the LD. (c) Alternatively LDs can expand by a ripening process called permeation. Here neutral lipids are transferred from a smaller LD to a larger LD. In adipocytes (d) Under certain conditions, large LDs can form by fusion/coalescence of two or more LDs.

2.1.5 Location and distribution

LDs frequently localise adjacent to the ER, mitochondria and peroxisomes, which likely reflects close functional interactions between LDs and these organelles (Fujimoto et al., 2008). The cytoskeleton plays a role in controlling LD distribution. Using live imaging, some LDs show a long-range directional movement along microtubules, which is consistent with the presence of dynein and kinesin-1 in LDs (Mason et al., 2014). Nevertheless, most LDs show a short-distance Brownian movement, suggesting that the LD distribution is also affected by factors like intermediate filaments (Franke et al., 1987; Heid et al., 2014) and other organelles (Wolinski et al., 2012).

2.1.6 Roles or functions

Foremost of LDs roles, is serving as important intracellular reservoirs of lipids. These lipids provide energy and serve as substrates for membrane synthesis, lipid trafficking, the growth of pathogenic viruses, protein storage and degradation; functions that established LDs as important organelle in the regulation of lipid homoeostasis (Wilfling et al. 2014, Albert et al. 2014, Yang et al. 2012). To elaborate the role of lipid storage in energy provision; it was estimated that an average 70 kg, non-obese man stores up about 2,500kJ of energy in the form of glycogen. Nevertheless, a substantial amount of lipid >500,000kJ, enough to run a marathon, is stored in the adipocyte in the form of triglycerides. LDs also participate in protein degradation, serving as a temporary storage site for unfolded protein before proteasome degradation (Olzmann et al., 2013). It has been shown that physiologically, ER stress can result from mutations of gene encoding glycosylation enzymes, which may be precipitated by elevated TGs and SEs in mutants compared to wild-type (Krahmer et al., 2013). The LD is as well pivotal to hepatitis c virus assembly and other pathogen infections (Herker & Ott, 2012; Kory et al., 2016).

2.2 Intramyocellular Triglycerides (IMTG) and the PLIN proteins

The LD surface is coated with many proteins, which regulate various aspects of LD biology. Research indicate that IMTG lipolysis is mediated by a protein-protein interaction occurring on the surface of the LD (Macpherson et al., 2013). Evidence from

proteomic and histological studies have identified many LD-associated proteins, ranging from; lipid synthetic enzymes, membrane trafficking proteins and proteins involved in protein degradation, such as Rabs and SNAREs, RalA and Rap1b. However, the perilipins (PLINs) 1-5 were the first proteins identified in eukaryotic LDs (Mason et al., et al., 2014). They are the major structural proteins on the surfaces of LDs. The PLIN proteins (perilipin/ADRP/TIP47), formerly called the PAT proteins, are expressed in several types of cell and tissue where they serve various important roles. PLIN1 in adipocytes and PLIN5 in oxidative cells such as the skeletal muscle, while PLIN2 and PLIN3 are expressed in a variety of cells and tissues, may act protectively against lipolysis (Ohsaki et al., 2014). PLIN expression has been shown to be influenced by sex, and IMTG level but only PLIN5 protein seem to increase with endurance training regardless of sex or age (Peters et al., 2012); suggestive of its vital role in LD homoeostasis. It is a popular opinion that PLIN 2, 3 and 5 play significant role in the regulation of skeletal muscle lipid lipolysis.

2.2.1 PLIN5

PLIN5, formerly known as OXPAT or LSDP5, is a major structural protein of intramyocellular LDs (Wang et al., 2011). PLIN5 has received a lot of research attention recently due to its promising role in the entire LD homoeostasis. It is also a variable of interest in this research. Therefore, more attention is paid to its characteristic features and functions in the domain of lipid metabolism and homoeostasis based on available research. PLIN5 has been shown to be expressed in oxidative tissues such as the skeletal muscle (Billecke et al., 2015; Bosma et al., 2012; Harris et al., 2015). It is localised on the lipid droplet surface and the cytosol of skeletal muscle cells. It is also localised in the SS region and near mitochondria (Bosma et al., 2012; Gemmink et al., 2016). Due to its proximity to mitochondria and LDs, PLIN5 is believed to interact with LD supporting fat mobilisation during lipolysis or beta-oxidation, thus mediating the transfer of lipid contents into mitochondria (Bishop et al., 2014; Wang et al., 2012; Wang et al., 2011). Some researchers suggest that this may be due to PLIN5 interaction with adipose triglyceride lipase (ATGL) during fat catabolism (Granneman et al., 2011; MacPherson et al., 2015, 2013). Consistent with this view, endurance exercise also appears to

upregulate PLIN5 expression. Endurance athletes expressed higher PLIN5 content relative to insulin resistant subjects with similar level of IMTG, which may be indicative of its role also in the oxidation of lipid content in skeletal muscle cells (Bosma et al., 2012; Gemmink et al., 2016; Kimmel & Sztalryd, 2014; MacPherson et al., 2012; Morton et al., 2016) The association of PLIN5 and skeletal muscle oxidative capacity is not clear cut. So far, a little research shows an association of PLIN5 content and increased oxidative capacity in the skeletal muscle tissues (Ramos et al., 2014; Bosma et al., 2013; Vigelsoe et al., 2015).

2.3 Genetics, health and physical performance

The place of genetics, health and physical performance is a continual subject of discussion. These factors are almost inseparable. However, if the influence of one over the other can be measured, it could help us to make informed decisions, design treatment or intervention for common diseases and pathological conditions, suggest or make a recommendation for enhanced physical performance and exercise. The knowledge of the interaction of these entities is therefore an important domain of science, especially in this age of heightened reduced activity level amongst diverse populations, and increased records of diseases with genetic undertone. Koch et al. 2013 identified two main divides that contribute to exercise performance as; 1) an intrinsic component that operates in the sedentary (untrained state) and 2) an extrinsic component that follows as an adaptive response that accrues from all activity above the sedentary state. Although a large part of physical exercise capacity is attributable to biological inheritance, extensive research supports the malleability of such capacity through increased and consistent physical activity (Alfieri et al., 2015; Karvinen et al., 2015; Park et al., 2016). The challenge, however, is the difficulty in separating the effects of genetics and exercise or the so-called environmental factor in everyone (Schwarzer et al., 2016; Stephenson et al., 2012).

This therefore reinstates that the mixture of genetic and environmental backgrounds contributes to the range of training-induced adaptations. The considerable challenge of identifying the mechanistic connection between exercise capacity and human health remained unresolved (Koch et al., 2013). Heritability of specific phenotype is often a crucial factor for probing a gene, and response to exercise or adaptation to the

environment is highly correlated to evolutionary fitness. One speculation and major view of the ETH is that evolution of biocomplexity is dependent on the capacity and efficiency of the use of oxygen from over 2 billion years ago and may relate strongly with complex diseases (Britton & Koch, 2001; Garton et al., 2016; Koch & Britton, 2007). Exercise has been shown to be a useful tool for improving health and physical performance, however, it appears that how much influence or effect this would have is dependent on genetic predisposition (Ren et al., 2013; Schwarzer et al., 2016). Exercise was shown to have no significant effect on insulin sensitivity inherent to genetic predisposition (Schwarzer et al., 2013) while a recent study reveals that both the genetic predisposition for exercise capacity and exercise training may affect recovery of cardiac function after Ischemia (Schwarzer et al., 2016). The interaction or influence of genetic and environment on issues like, metabolic disease (Lessard et al., 2011; Noland et al., 2007; Overmyer et al., 2015), aging (Karvinen et al., 2016), longevity (Koch et al., 2011; & 2012), diet (Kakehi et al., 2015; Van Proeyen et al., 2011) etc. also revealed interesting detail at the molecular level in support of some of the above stated views.

2.4. Exercise and IMTG

Lipid and carbohydrate serve as the major source of energy especially during physical exercise but also at basal state. An overview of the uptake of free fatty acid (FFA) from circulation into cells can be illustrated in four basic sequences (Wu et al., 2006); 1) localized generation of FFA through hydrolysis of TG-rich lipoproteins by lipases inside the endothelial lumen and binding of fatty acids to albumin, 2) fatty acid dissociation from albumin followed by binding to plasma membrane proteins or integration into the lipid bilayer, 3) their transport across the plasma membranes, and 4) their intracellular association with fatty acid binding and acyl-CoA binding proteins (FABPs and ACBPs, respectively).

Unlike with carbohydrates, not much advancement has been recorded in elucidating the mechanism and pathways involved in lipid metabolism, in the skeletal muscle, especially, during exercise. The main systems proposed for the transportation of FFA into cells or tissues are the classic passive and active substrate transport system (Bonen et al., 2007; Hamilton et al., 2001). The former is a simple diffusion, which depends on; FFA delivery,

aided by the concentration of FFA and blood flow, while the latter is a transport system mediated by FFA binding protein. Scientists are however sceptical of the plausibility of the simple diffusion proponent. Kampf et al. 2004 showed that the lipid bilayer of the plasma membrane would impede the FFA free diffusion, and tips off an alternative process that would take care of such inefficiency, (Kampf & Kleinfeld, 2004; Trimble & Grinstein, 2015). There is no doubt that some amount of FFA are likely transported via simple diffusion, but larger body of research have shown that the transportation of FFA is through highly regulated protein-mediated medium (Chabowski et al., 2006; Hajri et al., 2002; Luiken et al., 2002).

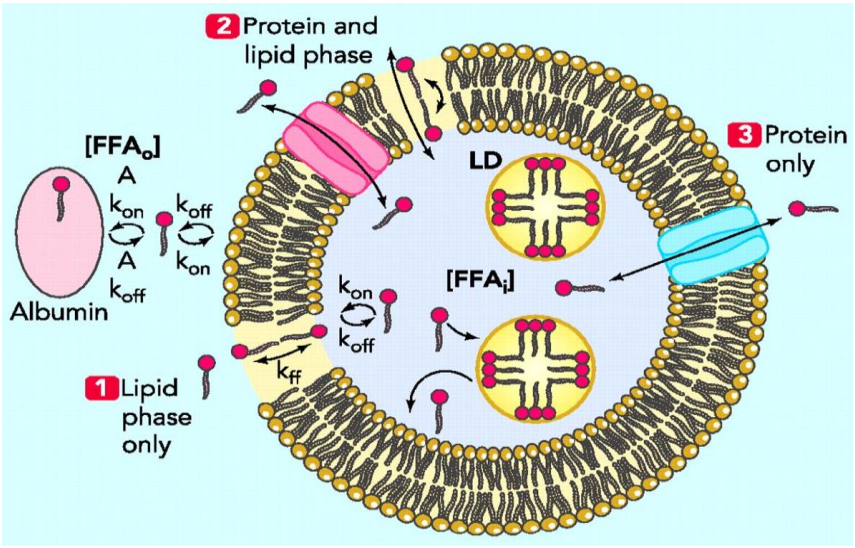

FIGURE 4. Illustrates the possible mechanism of FFA transports across the intracellular and extracellular aqueous phases. It depicts the diverse mechanism suggested by researches, (1) Simple diffusion of FFA across the lipid bilayer. (2) Protein mediated diffusion (3) complete membrane protein dependent transport of FFA. It also describes the reversibility of the reactions in both directions.

While the exact physical mechanism involved in the transportation of FFA across plasma membrane remains contestable (Kampf & Kleinfeld, 2007), unceasing attendance of research is elucidating the functions of identified proteins (FABPpm, CD36/FAT, FATP, and Caveolins) associated with FFA transport. According to Bonen et al. 2007, the process and rate of transportation of FFA may differ depending on the cell type (metabolic role or oxidative capacity). His research group working with giant vesicle of different

tissues, shows that FFA can transverse the plasma via passive protein-mediated transportation or on the other hand, an active protein-mediated mode of transportation that will require the input of energy (Bonen et al., 2007). He concluded however that, transportation of FFA into the heart or skeletal muscle does not appear to be energy consuming. FATPs were one of the first FFA transporter proteins identified to be responsible for the translocation of FFA, speculated to be a major FFA-transporter across plasma membrane especially in the adipose (Abumrad, 1999; Doege, 2006). A recent study with the advent of new measurement aid, give credence to FAT/CD36 as the main transporter of FFA. It is believed that it may play an essential part in the eventual oxidation and esterification of intracellular depots (McFarlan et al., 2012; Yoshida et al., 2013). The research however, downplays and casts doubt on the supposed roles of mitochondria in the biogenesis and oxidation of FFA but shares the idea of an interactive or coordinated function among the FFA transporter proteins.

The fate of the internalized lipid (in this case, IMTG) is absolutely another area that has received a lot of research interest, but contrasting opinions persist about LDs metabolism and the concurrent contribution of such depot to energy provision particularly during exercise (Kiens, 2006; Koonen et al., 2004; Watt et al., 2002). Once FFA is taken up by the active muscle cell it can be oxidised or incorporated into intracellular lipid-like membranes and/or re-esterified and stored into IMTG (van Hall, 2015). The contribution of substrates to energy production during exercise is a well-established phenomenon; substrates are derivable from both the extra and intramuscular locations (i.e. LD). Although the extra myocellular store (adipose and hepatic storage) is credited with the largest reserve of lipids, recent evidence suggests that IMTG may be responsible for substrate supply for contracting muscles (Watt et al., 2002). The lipolysis of IMTG has since been shown to be facilitated by the action of both insulin and muscle contraction.

2.4.1 Effects of exercise training on IMTG

The role of exercise intensity and duration in substrate metabolism and fuel selection is probably a well-defined term. For exercises of long duration and moderate to low intensity, more dependence is on lipid with a proportionate use of carbohydrate. During prolonged exercise and with the gradual depletion of carbohydrate the lipid substrate

takes prominence. However, carbohydrate serves as the major source of energy during activity of high intensity. According to Watt et al. 2002; IMTG and lipid stores located between myofibrils potentially provide an important substrate supply for a contracting muscle. TG consists of a glycerol backbone condensed with three fatty acyl-CoA molecules. During exercise, the complete breakdown of IMTG is thought to be overseen by two lipolytic enzymes, the ATGL and HSL. IMTG are stored as LDs within the cytoplasm of skeletal muscle cells near the mitochondria, where the FFA released via hydrolysis are ultimately metabolised. Endurance exercise has been shown to influence the number, size, location and redistribution of the IMTG pool. IMTG storage and perhaps utilisation is increased with endurance training. Endurance exercise was observed to affect the redistribution of LD from the IMF region of the cell towards the sarcolemma. Such redistribution has been shown to directly correlate with the rate of IMTG oxidation in the cells. It may be that the increased utilisation of IMTG during moderate to high exercises translates to the marked reduction in IMTG post exercise, observed in exercised subjects.

2.4.2 Association between IMTG and insulin resistance

Several factors could affect the availability of IMTG or the capacity to utilise them even when they are available. Training status and or insulin sensitivity are examples that have been researched by many studies, suggesting correlation between increased IMTG and insulin resistance (Bonen et al., 2006). Howbeit, observation from trained endurance athlete open another facet of a phenomenon. In trained athletes, elevated level of IMTG is accompanied by high insulin sensitivity. It has been shown that excess IMTG metabolite accumulation may interfere with insulin signalling and conversely impairs rates of fatty acid oxidation which are associated with insulin resistance (Bonen et al., 2006). Some studies have identified an elevated level of protein coat LD as a marked difference between a group of populations with similar prominent level of IMTG (i.e. endurance athletes and persons with a metabolic disease). They speculate that increased pool of LDs decorated with PLIN5 may represent an adaptive feature for maintaining LD dynamics and prevent insulin resistance (Gemmink et al., 2016). It was observed that biological characteristic of LDs (location, size, number of coat protein, proximity to

mitochondria etc.) other than number or quantity may be the thin line between the physiological and pathological consequence of LD depots observed among these populations (Bosma et al., 2013; Gemmink et al., 2016; Mason et al., 2014).

2.4.3 Capacity for oxidative metabolism

Oxidative capacity has been suggested to be primarily innate. Studies from selectively bred rats for high capacity and low capacity endurance exercise, however, indicated substantial physiological adaptation resulting from physical activity, especially at the SS and not limited to the functional capacity of the heart or lungs (Schwarzer et al., 2010; Wagner et al., 2002). Studies in which individual muscle fibers were dissected out after staining for myofibrillar ATPase revealed that IMTG was positively related to the oxidative capacity of the muscle fiber such that pooled type I muscle fibers contained greater IMTG compared with pooled type II fibers (Essén et al., 1975; Watt et al., 2002). Artificial selection for low aerobic treadmill running capacity demonstrated higher visceral adiposity, blood pressure, TG and insulin (Koch & Britton, 2001; Koch & Britton, 2008). Low aerobic capacity was associated with reduced skeletal muscle metabolism and impaired metabolic health.

Observation from skeletal muscle perfusion indicated a functional deficiency in substrates handling by skeletal muscle, reduced molecular signalling and lower mitochondrial content (Rivas et al., 2011). Relative to LCR, HCR efficiently oxidise FFA and branched-chain amino acids (BCAAs), sparing glycogen and reducing accumulation of short- and medium-chain acylcarnitine (Overmyer et al., 2015). Many studies have also suggested a strong link between reduced skeletal muscle oxidative capacity and insulin resistance in subjects with obesity, type 2 diabetes or both (Goodpaster & Wolf, 2004; Pischon et al., 2004; Schrauwen-Hinderling et al., 2007; Rivas et al., 2011). Capacity for oxidative metabolism rather than measurement of the amount of TG was opined to be a better predictor of insulin sensitivity (Bonen et al., 2006).

3 PURPOSE AND AIM OF THE STUDY

The aim of this thesis was to investigate the effects of exercise and genetics on skeletal muscle lipid metabolism, with a focus on the localisation and association of lipid droplets, mitochondria and PLIN5 in the gastrocnemius muscle of the HCR and LCR animal models with disparate exercise activities level. Low exercise capacity was shown to be a stronger predictor of morbidity and mortality relative to other commonly reported risk factors such as obesity and insulin resistance. (Koch et al 2016 ; Kokkinos et al. 2008 ; Kavanagh 2003 ; Myers et al. 2002). Quantity and or location of mitochondria, LDs and Plin-proteins in the skeletal muscle cells have been implicated in the onset of many metabolic diseases and may predict overall physical and functional capacity of an individual (Koch & Britton, 2008).

Although a large part of exercise capacity is attributable to biological inheritance, numerous studies support the malleability of such capacity through physical exercise (Alfieri et al., 2015; Green et al., 2013; Karvinen et al., 2015). The influence of exercise capacity and the concurrent effects of exercise activity level on the properties (number and location) of the identified particles form the basis of this thesis research questions.

3.1 Research questions and hypotheses:

1. What are the effects of exercise-induced changes on the skeletal muscle property and metabolism of individual with disparate genetic and exercise background?

   Hypothesis: Increased physical activity level is associated with higher number of LD, PLIN5 and mitochondrial content in the skeletal muscle tissues of HCR and LCR rats. LCR-R have higher LD, PLIN5 and Mitochondria content compared to LCR due to higher level of physical activity. HCR-R does not differ expressively from HCR despite difference in physical activity level due to similar high innate aerobic capacity. Voluntary running is associated with particle colocalization among exercise group.

2. What are the effects of intrinsic aerobic capacity on the skeletal muscle properties

and metabolism in individuals with disparate genetic and exercise background?

Hypothesis: HCR have higher number of LDs, PLIN5 and mitochondria particles compared to LCR due to higher innate aerobic capacity. HCR-R also differ expressively from LCR consequently, due to additive effects of innate capacity and exercise level.

4 METHODS

4.1 Setup

Samples. This experiment examined 40 gastrocnemius muscle (n = 10/group) samples of the HCR and LCR rat models. The samples were obtained from a previous study, and the experimental protocol consists of a speed-ramped maximal treadmill running tests to the rats as described previously (Koch & Britton, 2001; Karvinen et al., 2016).

Protocol. Rats were divided evenly into weight and maximal running capacity matched groups (n = 10) and randomly assigned to control (standard cage) vs voluntary running groups (cage with an access to a running wheel). Karvinen et al., 2016 give a full description of the protocol.

Sample collection. At the age of 9 and 21 months (after one-year voluntary running intervention), the skeletal muscle samples (gastrocnemius muscle) were collected at the same time points. Four different subgroups were adopted for this present study, comprising the HCR (control), LCR (control), HCR-R (runner) and the LCR-R (runner) rats. The distal part of gastrocnemius muscle (2/3 of the total muscle volume) was used for the analyses. Muscle samples were mounted in Tissue Tek O.C.T. embedding medium (Tissue Tek, Sakura Finetek Europe) and stored in -20 ℃.

4.2 Immunohistochemistry

Sample sectioning (10µm serial cross sections) was done using a cryostat (Leica CM3000, Germany) at -20ºC and the sections were collected on 13mm round coverslips. The sections were immediately fixed with 4% paraformaldehyde at room temperature (RT)and then washed three times for five minutes with 0.05% Saponin in Phosphate Buffer Saline (PBS-SAP). This was followed by blocking with 5% goat serum and 5% donkey serum in PBS at RT.

Primary antibody incubation was performed in 1% goat serum diluted in PBS-SAP. Sections were incubated for 1 hour at room temperature (RT) followed by 3 times

washing for 10 minutes each with PBS-SAP. The antibody references and respective dilutions are specified in Table 1.

The secondary incubation was done in a dark-moist environment for 2 hours at RT followed by washing 4 times for 10 minutes with PBS-SAP. The sections were then stained with neutral lipid stain, BODIPY 493/503 – 1 mg /ml ethanol, for 20 minutes at RT with 0.01mg/ml concentration in PBS-SAP and then washed briefly 2-times with PBS-SAP before mounting in Mowiol with 2.5% DABCO to prevent fading or photo-bleaching. The detailed outline of the dilution procedure is presented in Table 1.

TABLE 1. Summary of the primary and secondary antibodies and dilution concentration of the immunohistochemical staining.

Particles/ Staining	**Primary antibody**	**Species**	**Dilution**	**Secondary antibody**	**Species**	**Dilution**
Mitochondria (COXIV)	Abcam 153709	Rabbit	1:400	Abcam (98499) 555	Donkey	1:200
OXPAT	Progen (602081)	Guinea-pig	1:100	Jackson IR 549	Donkey	1:200
Fast Myosin	SIGMA MY32	Mouse	1:100	Alexa Fluor (A31553) 405	Goat	1:200

4.3 Microscopy

Microscopic images were collected with the ZEISS LSM 510 confocal microscope (Carl Zeiss, Thornwood, NY). Images were generated with a 40x NA 1.25 oil immersion objective (NEOFLUAR). Fluorophores were excited with the aid of Argon laser using 488 and 555 nm vawelenghts3, reflecting LD – green, Mitochondria – red and OXPAT – red. The images were collected with ZEN-2010 (black) software, ensuring uniform acquisition settings throughout the sample-imaging period.

4.4 Image processing and raw data extraction

The generated microscopic images were further processed with ImageJ (http://rsb.info.nih.gov/ij/) to better reflect the properties of the staining and to clearly map the characteristics of the sample stained. Manual adjustment was done to highlight relevant features of the images and to generate numerical data.

Image adjustment illustrating qualitative observations ensures that images are made distinguishable by colours and in some cases labels and symbols. Colour, brightness, sharpness and contrast were manually adjusted to ease readers understanding of the images´ observable features intended. This also ensures that pivotal characteristic of particle prominent in the image are made easily observable. *Preparation for numerical data extraction and quantitative analysis* ensures adjustment involving linear processing and merging of two or more channels for statistical parameters. Threshold, intensity localisation and colocalization of particles was assessed with the intensity correlation analysis method in line with Coast et al. 2004.

4.5 Statistics

Numerical data was extracted from the images collected as previously described. Statistical analyses for variables were completed with MATLAB (Pearson Education, 2004). The covariance between outcome variables in testing the statistical significance of the mean difference was tested using a two-way repeated measure of univariate analysis of variance (ANOVA). Statistical significance was established at $P \leq 0.05$. Data are presented as means ± SEM.

5 RESULTS

5.1.1 Numbers of lipid droplets and location in the skeletal muscle cell

LD particle across groups present interesting characteristics (Figure 5; A, D, G, and J). Staining intensity appears similar but particle distribution is lot more different across groups. The LCRs have cells ranging from large number of LD to cells with very minute number, and the LD particle in the LCR-R tissues (Figure 5; J, K, and L) seem to be more proportionately distributed across the cells.

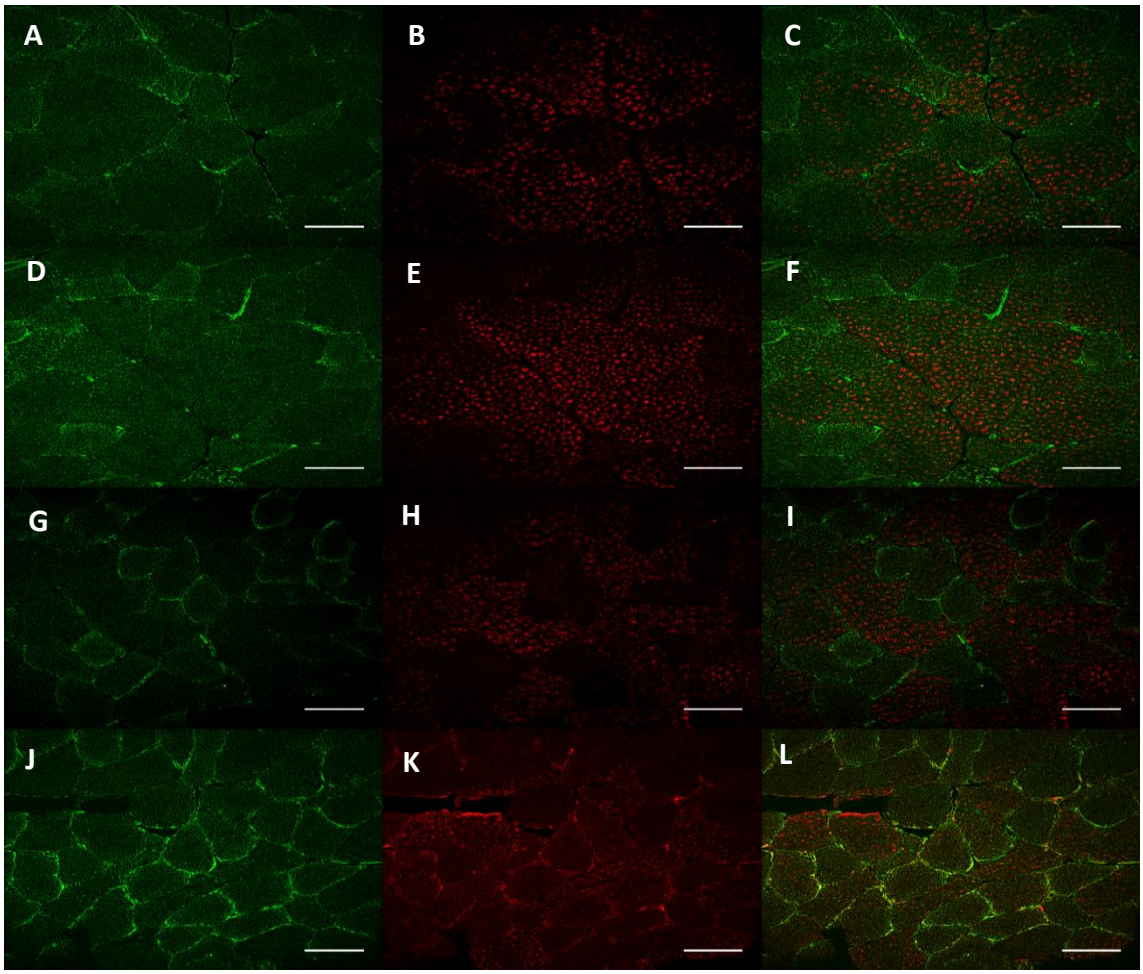

FIGURE 5. An example summary of immunohistochemical staining of mitochondria (red), LD (green) and merged sample (red and green) of the gastrocnemius muscle tissue of the HCR and LCR rats. HCR (A, B, C), HCR-R (D, E, F); LCR (G, H, I) and LCR-R (J, K, L). Sections immunoassayed for LD particles show a somewhat similar pattern of particle distribution, number and intensity between groups. Bar length 50µm

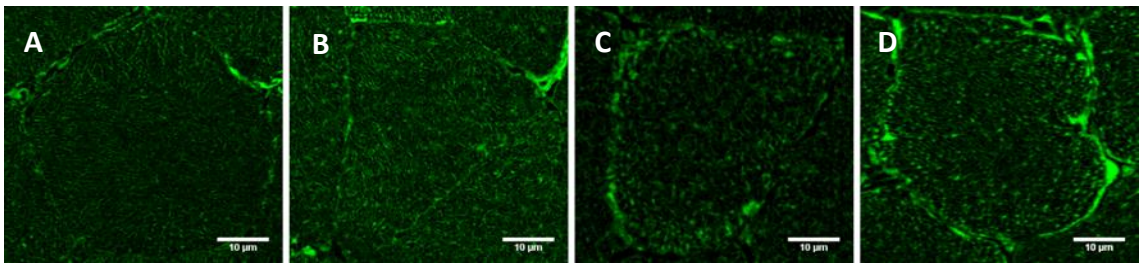

FIGURE 6. An example immunohistochemical LDs stain signal in the skeletal muscle cells of different rat groups. (A, B) HCR and HCR-R LD rich cells with disparate number across cells. LCR and LCR-R LD content (C, D); with the later appearing to have a better share of SS distribution compare to the other groups (Figure 6D).

5.1.2 Mitochondria number and location in the skeletal muscle cell

HCR and LCR groups shares similar intensity and perhaps distribution but there is a clear difference in particle contents between the LCR-R and the other groups. There is fair share of similarity in intensity between the LCR and the LCR-R. However, the number of cells with greater intensity are more in the LCR-R muscle tissue samples compared to the LCR sample (Figure 7C and D). Like the pattern observable between the HCR and HCR-R, the LCR-R muscle cells have greater number of particles signal in the muscle tissues compared to the LCR.

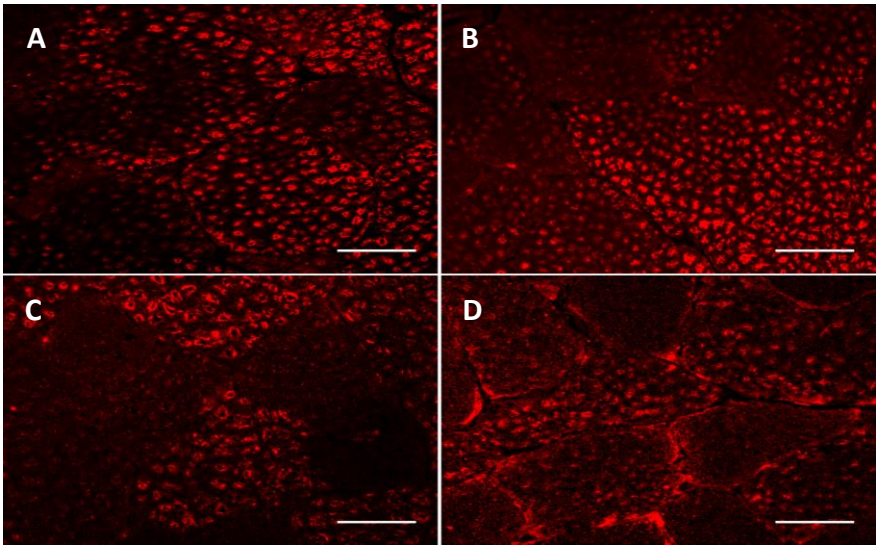

FIGURE 7. An example immunohistochemical mitochondria stain signal in the skeletal muscle cells of different rat groups. (A, B) HCR and HCR-R LD mitochondria content,

(C, D) LCR and LCR-R mitochondria content, with the later appearing to have more cells with mitochondrial stain signals compared to LCR group (Figure 7D). Bar 100µm

5.1.3 Numbers of PLIN5 and location in the skeletal muscle cell

The features of the PLIN5 particle are also quite fascinating, though distinction in characteristics such as size and particle distribution are difficult to observe, while intensity of stain and number of plin5 are probably more noticeable. The number of PLIN5 variability maybe noteworthy between the LCR and the LCR-R, with more cells in the LCR-R tissues having more stain. PLIN5 in the LCR distribution seems concentrated in selected cells but reduced in notable number of others (Figure 8). The idea is to compare the pattern of distribution particles on separate image.

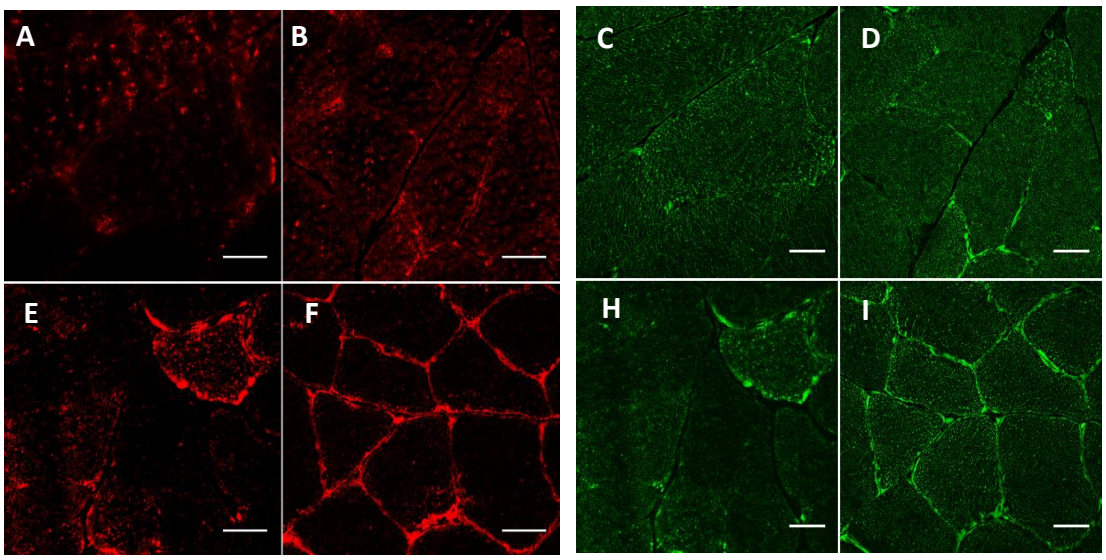

FIGURE 8. An example immunohistochemical PLIN5 stain signal in the skeletal muscle cells of different rat groups. (A, B) HCR and HCR-R LD PLIN5 content, (E, F) LCR and LCR-R PLIN5 particles, with the LCR-R appears to have more cells with PLIN5 stain signals compared to LCR group in more cells, maybe more at the SS region. (C, D) HCR and HCR-R LD particle of the (A, B) PLIN5. (H, I) LCR and LCR-R LD particles of the (E, F) PLIN5. Shows similarity in the number of LD and PLIN5 in the muscle tissues. Bar 100µm.

5.2 Colocalization of particles

5.2.1 Colocalization of LD and mitochondria

The mitochondria particles measured through the COXIV signal highlights the aerobic and physical functional capacity difference between the rat groups. However, colocalization seem to not to be as obvious as the number of mitochondria particles between the groups. Through, the runner groups (LCR-R and HCR-R) appear to have higher number of particles in more cells in most tissues compare to the sedentary groups LCR and HCR, there is no notable difference in the interaction of the particles among these exercise and genetics disparate rat samples. What is, however, notable and common across the groups is the characteristic and somewhat rare colocalization in some points in the cell. In addition, some particles are closely located, such that LDs and Mitochondria particles seem to be located close to each other. Other form of observable signal characteristic comprises mostly scattered or un-colocalized particles located in different point in the cells hemisphere.

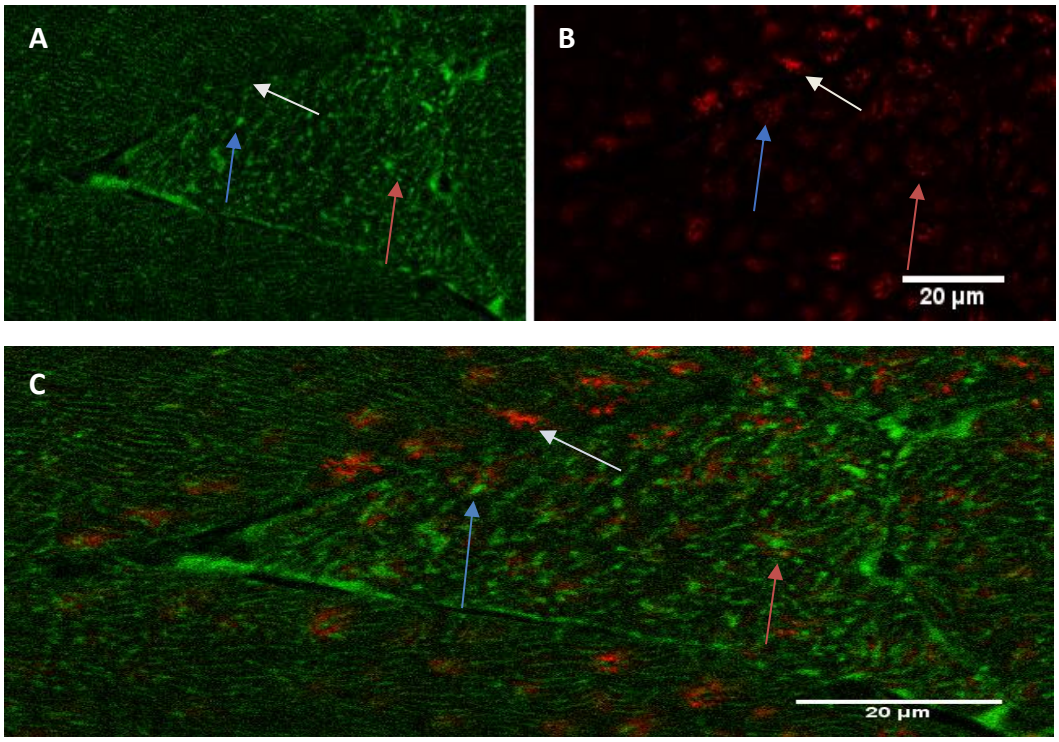

FIGURE 9. Example immunohistochemical mitochondria and LD stain signals. (A, B) LD and mitochondria stains with three pointing arrows (white, blue and orange), (C)

merged images of LD and mitochondria signals. White arrow pointing example of uncolocalised mitochondrial particle, Blue show particles of LD and mitochondrial marker stain next to or nearby each other. While the orange arrow point stains that appear like the LD particles are wrapped by the mitochondria stains. Bar 20µ

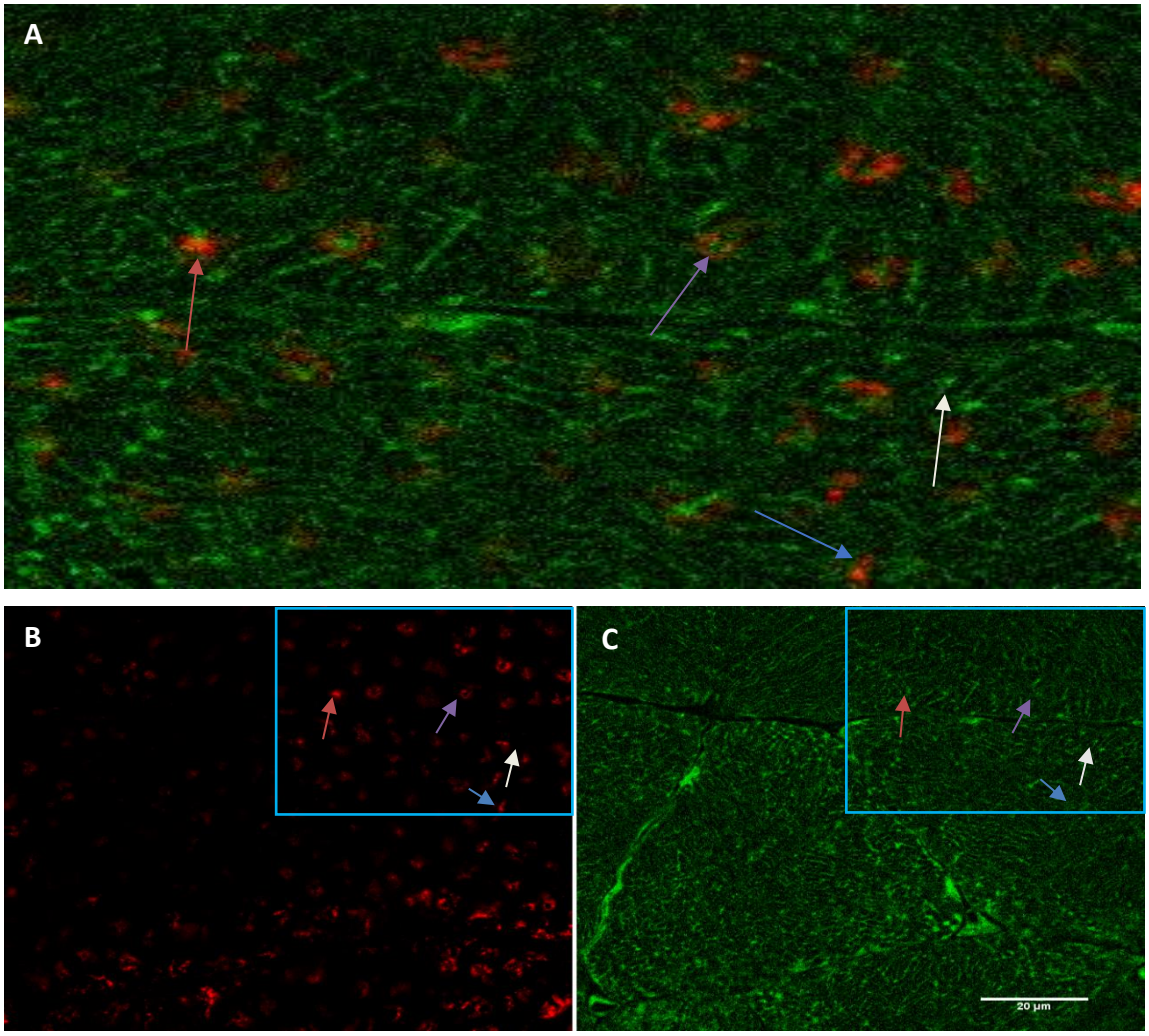

FIGURE 10. Example merged immunohistochemically stained mitochondria and LDs. (A) Incept from images B and C, showing the merged images of LD and mitochondria signals with four pointing arrows (white, blue, purple and orange). (B, C) LD and mitochondria stains. White arrow pointing an example of uncolocalised LD particle, Blue point at an example of free mitochondria, yellow arrow shows particle of LD and mitochondrial stain next to or nearby each other. While some rather rear cases (orange arrow), it appears as if the LD particle really colocalized with the mitochondria marker signal. Bar 20µ

5.2.2 Colocalization of LD and PLIN5

The colocalization and perhaps the interaction between PLIN5 and LD particles between the rat groups does not seem to differ greatly. However, some common pattern of colocalization exist across the groups. Many obvious and strong LD signals appear completely alone without equivalent PLIN5 signal close to them. In some cases, PLIN5 and LD particles seem to be at a close range or side by side, but not particularly sharing a location or microscopic point to be considered exact colocalization or strong interaction. However, real colocation and perhaps strong interaction between PLIN5 and LD particles are also observable across the groups. Few particles of PLIN5 could be seen encircling the LD particle in some instance in a unique point or location in in the cells.

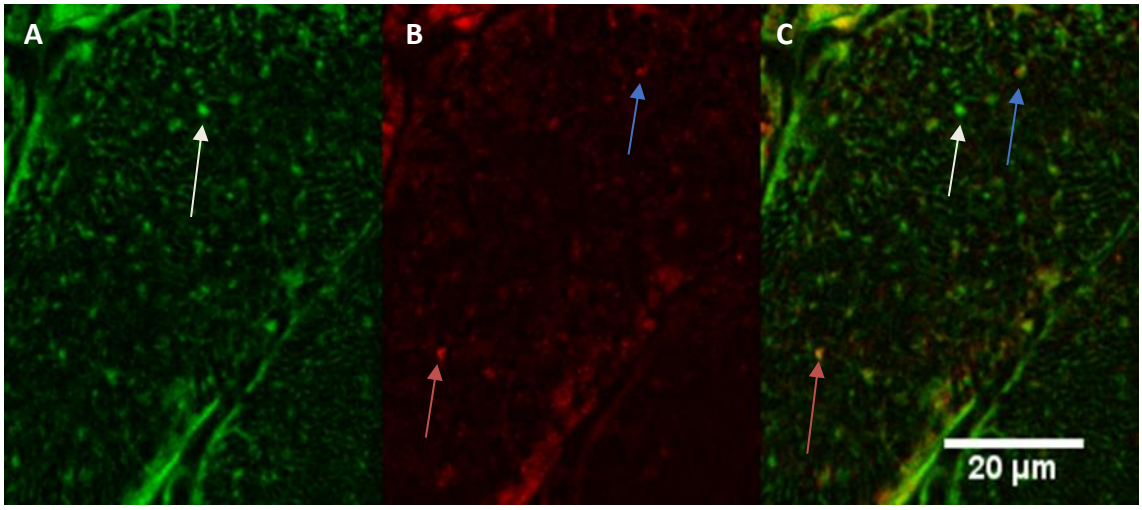

FIGURE 11. Example merged immunohistochemically stained LD and PLIN5 particles. (A, B) LD and PLIN5 stains with three pointing arrows (white, blue and orange), (C) merged images of LD and PLIN5 signals. White arrows point examples of uncolocalised LD particle, Blue show particles of LD and PLIN5 marker stain next to or nearby each other. While the orange arrow stains appear like the LD particles are enveloped by the PLIN5 stain signal (C). Bar 20µ

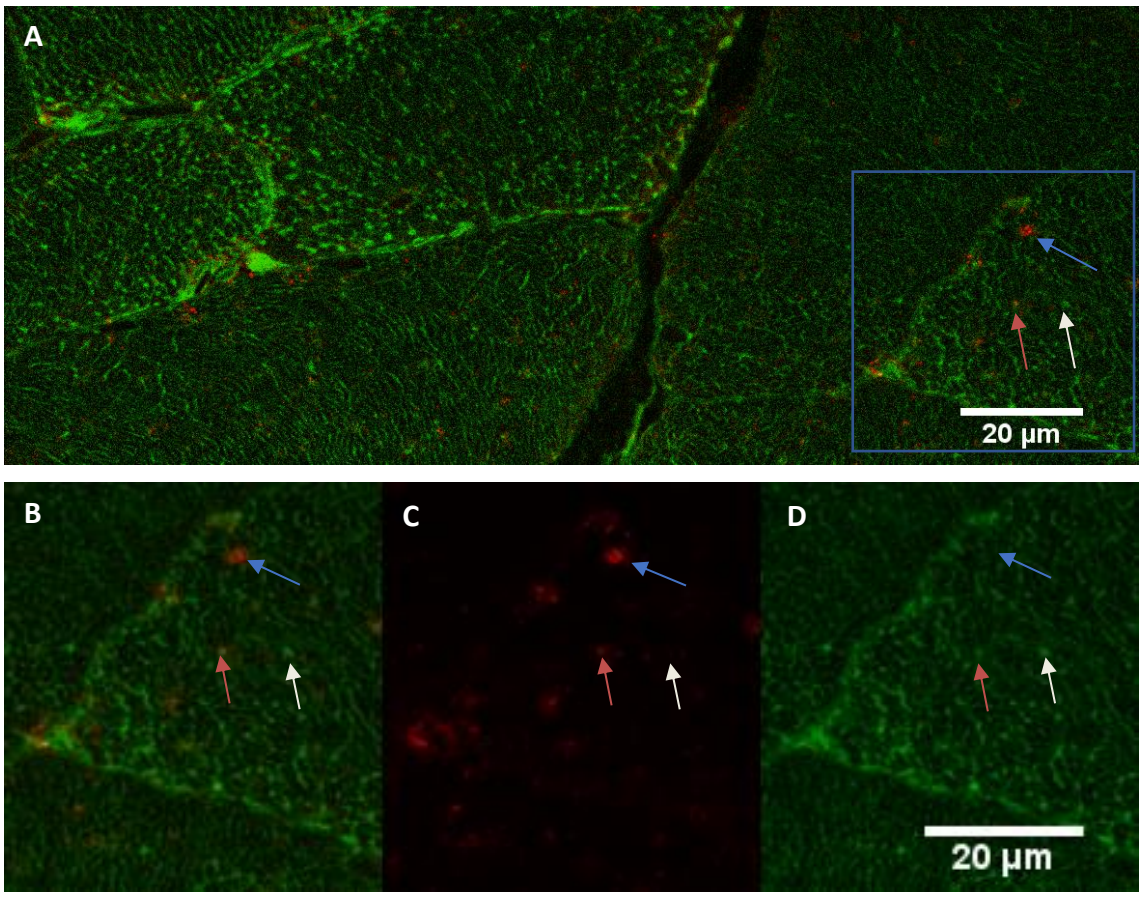

FIGURE 12. Example merged immunohistochemically stained LD and PLIN5 particles. (A) Shows merged images from LD and PLIN5 stain signals, (B, C, D) Incept from image A. Showing (B) the merged images of LD and PLIN5 signals, (C and D) represent PLIN5 and LD stains respectively. Blue arrow points at an example of free PLIN5 ring like particle, orange arrow shows particle of LD and plin5 stain next to or nearby each other. Bar 20µ

5.3 Animal weight and running data

Body mass and gastrocnemius muscle mass (relative to body mass) of the studied rat groups are listed in Table 2. Before the intervention, HCRs had lower body mass and higher relative gastrocnemius muscle mass compared to LCRs (line effect $p < 0.001$). However, HCR-R had higher body mass and higher gastrocnemius muscle mass compare to HCR (sedentary) after the intervention. LCR-R had lower gastrocnemius muscle mass (relative to body mass), but higher body mass compared to LCR (sedentary) after the intervention (Modified from Karvinen et al. 2016).

TABLE 2. Background information for immunohistochemical staining

Group	Body mass (g)	Gastrocnemius/Body mass (mg/g)
HCR_before	232 ± 30	5.32 ± 0.65
HCR_after	260 ± 36	4.57 ± 0.58
HCR-R_after	268 ± 34	4.78 ± 0.49
LCR_before	302 ± 26	4.73 ± 0.43
LCR_after	320 ± 37	4.21 ± 0.45
LCR-R_after	345 ± 48	4.00 ± 0.59
P-value	Line < 0.001***	Line < 0.001***

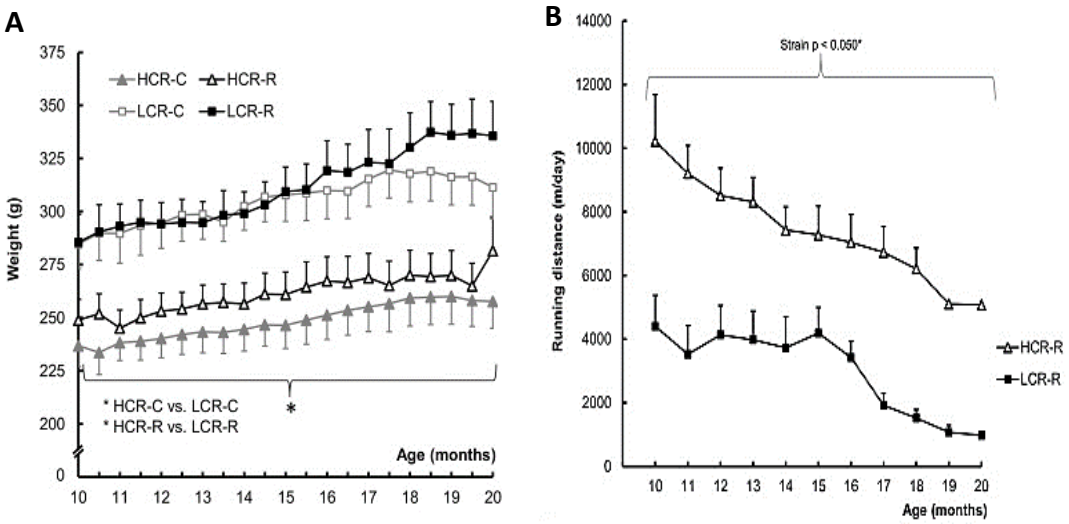

FIGURE 13. subject parameter during intervention (generated from previous study) (A) Body weight (B) Running distance during one-year intervention. n = 10/group. *, #p < 0.05. Values are expressed as mean ± SEM (ibid.)

5.3.1 Intensity analysis

A one-way between subject analysis of variance (ANOVA) was conducted on the number of LD, PLIN5 and mitochondria content across the group. The analyses revealed significant differences in the number of PLIN5 and Mitochondria particles across the groups. However, no notable difference or relationship existed in the number of LD particles and colocalization and or interaction between the LD and PLIN5 particles. There was no significant colocalization between the LD and the Mitochondria particle across the rat sample groups.

The intensity analysis of the LD particles (Figure 14) reveals similar number of LD across groups. The lower body mass and higher gastrocnemius/body mass ratio of the HCRs (Table 2) do not significantly affect the amount of IMTG in the skeletal muscles of the rat. There was not significant difference LDs across rat groups despite significant higher body weight of the LCRs group (Figure 13A).

Source	SS	df	MS	F	Prob>F
Groups	40.778	3	13.5927	0.43	0.7305
Error	751.369	24	31.3071		
Total	792.147	27			

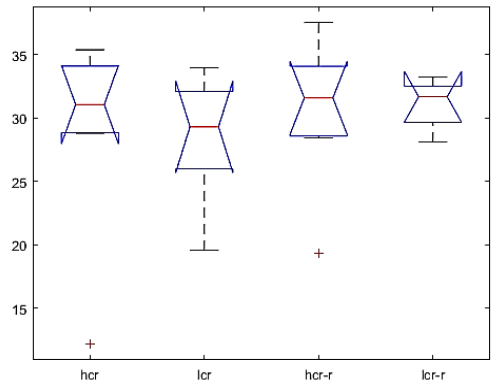

FIGURE 14. A one-way between subjects' ANOVA was conducted on the number of LD signal. There was no statistically significant difference in the LD signal across groups ($F(3,24) = 0.43$, $p>0.05$).

The number of mitochondria (CoxIV) particles is significantly higher in the exercise rat groups (Figure 15). The level of activity of the rats (Figure 13B) is relative to the enhanced mitochondria content observable in the intensity analysis.

Source	SS	df	MS	F	Prob>F
Groups	110.978	3	36.9925	4.24	0.0154
Error	209.287	24	8.7203		
Total	320.264	27			

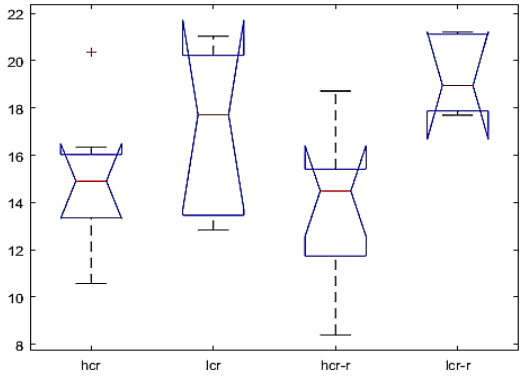

FIGURE 15. A one-way between subjects' ANOVA was conducted on the mitochondrial signal. This revealed a statistically significant difference in the mitochondria content across groups ($F(3,24) = 4.24$, $p<.0.05$). The LCR-R group expressed the highest number of mitochondrial marker compared to the other groups.

There was notable increase in the number of Plin5 particles in the exercise group (Figure 16). The increased number of the Pline5 also mirrored the pattern of increased mitochondria particles measured in the same sample groups (Figure 15) and relates to the running distance or activity level of the group (Figure 13B).

Source	SS	df	MS	F	Prob>F
Groups	170.636	3	56.8786	3.8	0.0231
Error	358.874	24	14.9531		
Total	529.51	27			

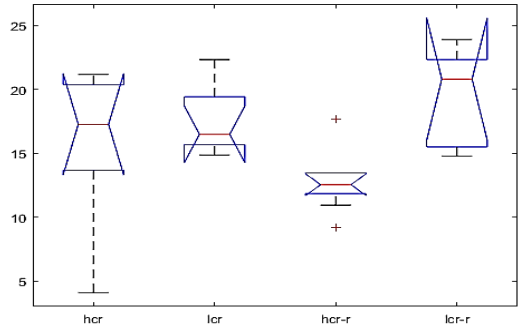

FIGURE 16. A one-way between subjects' ANOVA was conducted on the PLIN5 signal. This revealed a statistically significant difference in the proportion of PLIN5 across groups ($F(3,24) = 3.8$, $p<.005$). The LCR-R group expressed the highest number of PLIN5 marker compared to the HCR-R group, which had a lower PLIN5 expression.

5.3.2 Colocalization and interaction of particles

The increased number of Plin5 and Mitochondria particles (Figure 15 & 16) tend not to influence the particle colocalization. There was no observable difference in the particle colocalization of samples across the group (Figure 17 & 18).

Source	SS	df	MS	F	Prob>F
Groups	0.24935	3	0.08312	1.2	0.3307
Error	1.66125	24	0.06922		
Total	1.9106	27			

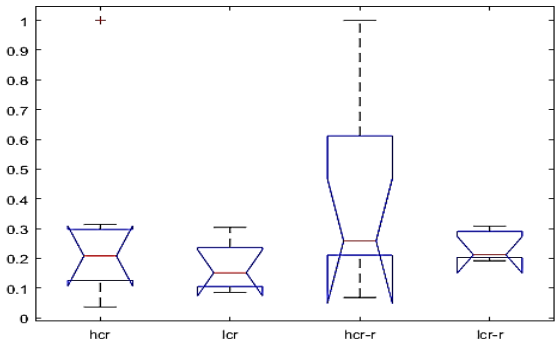

FIGURE 17. A one-way between subjects' ANOVA was conducted on the Cox IV and LD signal colocalization. There was no statistically significant difference in the in the

colocalization of particles across groups (F (3,24) = 1.2, p>.005).

There was no measurable difference in the Plin5/LD particle colocalization across the group. The observable increase in the Plin5 particles of the exercise group tend not to influence particles colocalization in the skeletal muscles of the rat samples.

Source	SS	df	MS	F	Prob>F
Groups	0.03068	3	0.01023	1	0.4078
Error	0.24434	24	0.01018		
Total	0.27503	27			

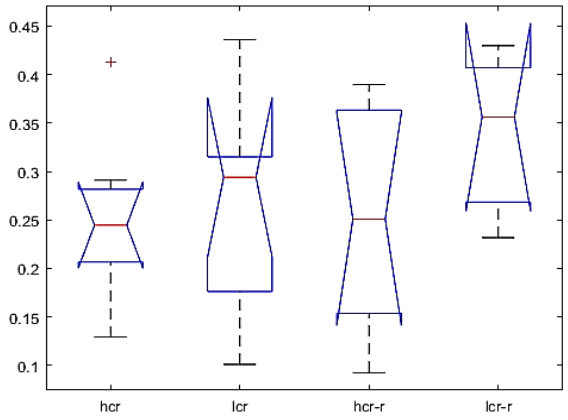

FIGURE 18. A one-way between subjects' ANOVA was conducted on the PLIN5 and LD signal colocalization. There was no statistically significant difference in the in the colocalization of particles across groups (F (3,24) = 1, p>.005).

6 DISCUSSION

This experimental model presents a unique opportunity to study this phenomenon in HCR and LCR rats with disparate aerobic capacity and physical activity level. Therefore, making it possible to study this paradox in the highly trained and sedentary, to elucidate the training or the genetic component of this metabolic phenomenon. The chemistry of the IMTGs or LDs and its contribution to cellular homoeostasis was the key area of interest of this study. The effective synthesis and hydrolysis or otherwise of LDs have been implicated recently in many physiological and pathophysiological functions. Paradoxically however, similar physiological characteristics were observed in the skeletal muscle tissues of individuals with common metabolic diseases and high performer endurance; both groups having relatively larger number of LDs in their skeletal muscle tissues (Dubé et al., 2008; Goodpaster et al., 2001). However, the functional and health implications seem to differ greatly (Gemmink et al., 2016).

Muscle contraction is one of the important mechanisms that have been identified to be responsible for the passive translocation of LDs, which is observed to be facilitated by the protein filament and microtubules for eventual oxidation in the mitochondria (Mason et al., 2014). One of the major assumption of this study is that; genetics background associates with the number of LDs, PLIN5 and mitochondria particles in the skeletal muscle cells. We speculate that metabolic functions relate to physical activity level, enhancing the quantity and more so the quality of the particles and relationship between them. We assume that the quality of protein coats, interaction with mitochondria and IMF or SS distribution of the LD is pivotal to its utility and determines the overall turnover during the metabolic process.

6.1 Hypothesis and research result

The differential roles of genetic background and physical activities on skeletal muscle property, metabolism and metabolic disease are yet to be clearly stated (Thyfault & Morris, 2017). Many studies have shown that the metabolic differences between HCR and LCR was tied to divergent skeletal muscle phenotypes (Bernal-Mizrachi et al., 2006;

Morris et al., 2016). Previous studies have revealed variability in the skeletal muscle myosin heavy chain composition, muscle capillarization, mitochondria content and efficiency. Some of these variations are linked to the cardiorespiratory and neuromuscular function of the rats; blood oxygen carrying capacity, oxygen delivery and extraction, pulmonary diffusion, cardiac output, skeletal muscle oxygen utilizing capacity, difference in fuel selection, efficient oxidation of fatty acid and branched chain amino acids. (Bassett and Howley 2000).

The present study inquires further into the variation in the skeletal muscle properties of the HCR and LCR rats. Immunohistochemical measurement of mitochondria contents, PLIN5 and LD particles in gastrocnemius muscles of the rats revealed that despite the differences in the physical characteristics of the rat groups (body mass, relative muscle mass, and physical activity level; (Table 2)) as stated in previous study (Karvinen et al. 2016). There was no significant difference in the number of LD particles in the skeletal muscle cells of the rats' tissues (Figure 14). Consistent with the previous result (Turner et al. 1997), mitochondrial content varied significantly among the rat groups, with major variation observable among the LCR-R, HCR-R and the HCR (Figure 15). In resonance with some reported outcome from similar research (Bosma et al., 2012 & 2013), PLIN5 particle was significantly higher in the LCR-R compared to the HCR-R (Figure 16). However, the interaction among the particles in the skeletal muscle cells across the group was not obvious (Figure 17 & 18). There was no significant colocalization of the LD and PLIN5 particles (Figure 17) nor were there substantial colocalization between the LD and the mitochondria particles across the rat groups (Figure 18). Descriptive observation however reveals some pattern of interaction, especially among the LD and PLIN5 particles (Figure 11 & 12), but the feature was not consistent across most tissue samples.

6.1.1 Number of particles

In this study we examine the mitochondria, PLIN5 and LD particles. LD particles are mostly found in the ER of the skeletal muscle cells, where they are believed to serve as substantive substrate for fuel or energy for the muscles especially during exercise or physical activity (Choudhary et al., 2016; Mishra et al., 2016). The abundance of the particles could, therefore, be beneficial as it supports high performer aerobic function and

participation in a long duration activity, serving as a reliable source of fuel (Shepherd et al., 2013). On the other hand, LDs were observed to be abundant in the skeletal muscle tissue of the obese and persons with metabolic diseases. In those cases, excessive storage of LDs is suggested to be harmful due to dysfunctional metabolic pathways (Stephenson & Hawley, 2013). The mitochondria are a unique cell organelle with a simple rod-like shape but complex structural morphology.

The size, shape, and structural organization of mitochondria, as well as the number and their intracellular location depend largely on the organism, tissue, and physiological state of the cell in question (Glancy et al., 2015; Suzuki et al., 1982). In this study, we examined the location and number of mitochondria by staining for cytochrome c oxidase (COX IV), a large transmembrane protein complex found in the mitochondria of eukaryotes. It is the last enzyme in the respiratory electron transport chain of mitochondria and is regarded as one of the major regulation site for oxidative phosphorylation. Such characteristics that suggests the relationship between aerobic capacity and the number of LD particles in the muscle cells (Overmyer et al., 2015). As observed in this study, mitochondria content was significantly higher in the exercise groups (Figure 15) and this is also reflected in the level of activity of the rat samples measured by their running distance in the free running wheels (Figure 13B). However, the significant difference in the running distance of the LCR-R and HCR-R group (Figure 13B) do not seem to introduce substantial dissimilarity in the number of the mitochondria between the group.

It is not surprising therefore that in the present study there was no significant difference in the content of the LD across the rat groups (Figure 14). Each group may have satisfied at least one characteristic factor such as metabolic dysfunction, high innate aerobic capacity or high aerobic physical activity level; factors which are believed to have close association with higher number of LD particles in the muscle tissues. The HCR have high innate aerobic capacity, HCR-R combine the innate aerobic capacity and physical activity level. The LCR group seem to possess the characteristic of the metabolic dysfunction, while the LCR-R might have gained substantial muscle properties adaptation due to the voluntary running, thereby acquiring aerobic capacity property characterized with increased number of LD.

It is important to consider the rats muscle mass difference relative to the body mass (Table 2). This parameter was reported to be significantly higher from the baseline and after the intervention in the HCR line compare to LCR. However, the significant muscle size difference between the rat line does not seem to significantly influence the number of LD in the skeletal muscle tissue of the rat groups. Since efficient fuel selection has been widely reported to be characteristic of HCR rats' innate aerobic capacity status (Kivela et al., 2010, Lessard et al., 2011). The HCR line are believed to have better capacity to metabolize fat, which serves as readily source of fuel that is promulgated by increased capacity for fatty acid oxidation and ensures enhanced LD turnover in their skeletal muscle.

Unlike the storage and reduced LD turnover reported in the LCR line, that may have foster equivalent number of LD in their muscle tissue despite its relative smaller size (Lessard et al. 2009). It is difficult to align the above assumption for the justification of LD content with notable adaptations and significant increase in the mitochondria and PLIN5 particles observable in the LCR-R compared to the HCR groups (Figure 15 and 16). A probably more logical assumption could be the reported effects of aerobic capacity and level of physical activity on the magnitude of adaptation from aerobic exercise (Lessard et al., 2011), which may have suggested the significantly higher adaptation in the LCR-R compared to the other groups (Jones & Carter 2000).

It is a common claim that aerobic capacity status may influence the level of adaptation, such that reduced level of capacity seem to promote higher response to exercise training (Timmons et al., 2010; Koch et al., 2013). Other studies (Bosma et al., 2013; Wang et al., 2011), also show that critical features of the skeletal muscle metabolism that are diminished in the sedentary LCR relative to HCR can be retrieved by exercise. They suggest that, while there appear to be similarity in the number of LD irrespective of metabolic status, disparate mitochondrial and perhaps PLIN5 contents would support the idea of a possibility in differences in IMTG turnover (Bosma et al., 2013; Wang et al., 2011)

6.1.2 Particle colocalization

The number of particles in the cells of skeletal muscle is in most cases directly proportional to the level or degree of functionality. In some cases, like the particles of LD, mitochondria or PLIN5, a higher number may not necessarily mean a better quality or greater level of metabolic capacity (Kakehi et al., 2015). The key to physiological function is mostly dependent on the properties of the particles that support interactions with complementary particles towards promoting the cell function (Kimmel & Sztalryd, 2014; Mohktar et al., 2016).

In this study, there was an observable adaptation from the exercise intervention on the increased number of particles in the skeletal muscle tissues of the exercise group, most significantly in the LCR-R group (Figure 16 and 17). Surprisingly and contrary to what we hypothesized, there was no significant group differences concerning colocalization between the particles in this study (Figure 17 and 18). Colocalization of particles describes a positional overlap that is interpreted as close association or interaction between the particles sharing such common spot. This form of interaction has been shown to depict or dictate in many cases the physiological function of the particles or sometimes the functional characteristic of the entire cell or tissue (Gemmink et al., 2016). It is known that PLIN5 appear to have a close relationship with LD and has been shown to interact with this organelle, helping to channel it into the site of metabolic beta-oxidation in the mitochondria (Kimmel & Sztalryd, 2014). However, the influence of the quantity or quality of PLIN5 in carrying out this function is not clear-cut (Mohktar et al., 2016).

The mitochondria are a well-known organelle of substrate metabolism, a function that has earned it the name the powerhouse of the cell. Many studies have shown the implication of mitochondria interaction with LD with contrasting views (Stephenson & Hawley, 2013). Such interaction however, calls for closer observation to elucidate probable difference that may support some previously research claims, especially in such unique groups of subjects as the HCR and LCR. In this study, no significant difference was found in the colocalization of PLIN5 particle and LDs between the studied groups (Figure 17 and 18). However, the characteristic colocalization of PLIN5 and LDs between the HCR-R and LCR-R seem distinct. This may be due to the substantially higher number of

individual particles found in the muscle cells of this groups compared to the other group (Figure 5). It is noteworthy however, that such features are in no means consistent across the samples.

6.1.3 Genetics and skeletal muscle properties

Taking a cue from the discussion in the literature review, extensive experimental programmes are elucidating the relevance of ETH or the genetic component of performance, with supportive research outcomes from many previous HCR/LCR researches, showing the performance and health variability between the two aerobically disparate rat groups (Novak et al. 2010; Kelly et al. 2010). In the present study, there was no clear difference in the colocalization of LD and mitochondria particles (Figure 9). The interaction between LD and PLIN5 particles was not also significant across the rat groups (Figure 5). However, number of PLIN5 and mitochondrial particles seem to segregate across rat line due to genetic influence (Figure 16).

There was observable difference in the number of mitochondria and PLIN5 particles in accordance to level of activity irrespective of genetic background (Figure 16). The skeletal muscle properties that are related to the aerobic function are polygenic, therefore the divide between the innate and acquired aerobic functions may be difficult to measure. However, this sample group come close to an idea approach to shed a little light on the phenomenon. Since highly fit individual or individual with high aerobic capacity are usually regular exerciser, it is often hard to conclude if the measured variables are due to innate capacity or activity level (Thyfault and Morris 2017). But the observable increased mitochondria content and Plin5 particles in this experiment may be narrowed to exercise effects. This also support a similar report on cardiac muscles, which shows that a genetic predisposition for high and low exercise capacity may determine aerobic exercise training effect on cardiac metabolism (Schwarzer et al. 2016). In a similar research, high or low aerobic exercise capacity phenotypes influence capillarity and skeletal muscle enzyme properties in the aerobic disparate rat group (Schwarzer et al., 2016)

6.1.4 Exercise and skeletal muscle properties

Many studies have reported the skeletal muscle adaptation to physical activities. Exercise has been shown to augment the number of LD particles, increase mitochondria content and efficiency (Ramos et al., 2015b; Shepherd et al., 2013; Wang et al., 2011). Some studies also report the increase in the number of PLIN5 because of regular moderate aerobic activity (Ramos et al., 2015b). The capacity of exercise to promote the aerobic capacity and function has been described by some studies relative to the cellular changes in the number and structure of particles in the skeletal muscle cells (Layec et al., 2016). Cellular studies (Kuznetsov and Margreiter 2009) show that mitochondria in skeletal muscle cells form a specialized network by joining or separating from nearby mitochondria in processes known as fission and fusion. These processes are essential for maintaining mitochondria functions and integrity.

There is clear indication that exercise regulates both fusion and fission, modulates important signals for the maintenance of mitochondrial network (Booth et al., 2015; Kivela et al., 2010). In the present study, LCR-R cellular adaptation to voluntary running is also notable (Figure 16 and 17). Consistent with previous studies, exercise seem to promote the number of mitochondria and PLIN5 in the skeletal muscle tissue of these aerobically deficient rats (Ramos et al., 2015). The implication of exercise intervention on the quality vis-à-vis the interaction between particles was not significant in this study. However, the increase in the number of particles may also suggest closeness of particles and perhaps improved interaction and biological interplay, but this remain very speculative. Some studies have suggested such possibility and observed similar changes in the skeletal muscle tissues following an aerobic exercise intervention (Bosma et al., 2012 & 2013; Wang et al., 2011).

In addition, similarity in the skeletal muscle ceramide and diacylglycerol content, basal AMP-activated protein kinase (AMPK) activity, and basal lipolysis were reported in the LCR and HCR in a previous study (Lessard et al., 2009), may also corroborate this similarity. Since such similarity in physiological function may poise one to look at other possibilities such as, similar physical structure or properties that may include, the intracellular binding protein, that has also been reported in other research as likely thin

line, setting the divide between aerobic functional capacity. One plausible assumption from the result of this study will be that, both increased physical activity level and metabolic diseased state seem to increase the total IMTG and percentage body fat or relative muscle mass. However, the former increases the number of mitochondria and or PLIN5 particles, which invariably may enhance FA oxidation and total LD turnover.

6.2 Study limitations

It is important to state at this point that there are number of limitations that has hindered some possible features and observable assumptions. We did not stain for the cell membrane of the tissues, a feature that can give more clarity to the boundaries or show more distinct shape and component of the individual cells. This could have ensured a more reliable observation of the distance of the particles from the sarcolemma for instance. In addition, spelling out the fiber type composition and distribution in the muscle tissues across the groups would have given some useful insight into the implication of myosin heavy chain composition between the groups. But the muscle sections stained seem to have too fewer type 1 fiber significant enough to be included in the parameter measured for analysis.

In addition, the quality of the muscle samples is also worthy of mention. Perhaps at the point of collection due to unascertained collection or storage circumstance, some of the tissues stained are not at the best possible shape or condition. However, samples imaging was performed with a confocal microscope, which ensures a more controlled, and accurate exposure time and illumination intensity across the imaged samples. Also at the point of acquisition, section imaged were sometimes not evenly illuminated due to poor sample quality, possibly having lost its antigens. However, the experimental procedure having followed standard processes and unwavering experimental protocols present some useful insight into the characteristic of the studied assumption within the spare of the manifest limitations.

7 CONCLUSIONS

The result suggest that genetic background may dictate the skeletal muscle properties at the cellular level (Koch and Britton 2001). The effect of genetic background may not only affect performance component but also health component of physical fitness (Koch et al., 2012). However, environmental influence such as physical activity, aerobic training (voluntary running) may reverse the deleterious effects of dysfunctional genetic trait and improve both performance and health characteristics of an individual (Lessard et al., 2011). The implication of exercise on the skeletal muscle structure, integrity and function of an individual with high innate aerobic capacity may not be as significant compared to the aerobically deficient individual (Karvinen et al. 2016). It appears likely that increased mitochondria content and concurrent increase in PLIN5 particle following aerobic exercise adaptation is related to enhanced aerobic capacity in the LCR-R rats.

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