ABSTRACT

Title of Thesis:	THE EFFECTS OF AEROBIC EXERCISE TRAINING AND INSULIN RESISTANCE ON LIPID DROPLET SIZE AND PERILIPIN PROTEIN ASSOCIATION
	Evan Ryan Bota, Master of Arts, 2026
Thesis Directed By:	Steven J. Prior, Ph.D., Associate Professor, Department of Kinesiology

Skeletal muscle insulin resistance has been related to impaired glucose clearance, lipid accumulation, and chronic low-grade inflammation in type 2 diabetes mellitus (T2DM). Growing evidence suggests lipid droplet (LD) morphology and LD protein association may explain the pathology contributing to insulin resistance. In T2DM, surface proteins inhibit the utilization of large LDs, which can further impair insulin signaling and reduce skeletal muscle glucose uptake. Aerobic exercise (AEX) training has been shown to improve skeletal muscle oxidative capacity and trained skeletal muscle contains smaller LDs with a distribution of perilipin proteins favoring effective lipid utilization. The aims of this thesis study are to address gaps related to LD morphology and perilipin localization in human skeletal muscle through visual quantification and total muscle protein expression. The outcomes of this thesis will enhance the current understanding of how impaired glucose regulation influences lipid metabolism in skeletal muscle while highlighting the effects of physical activity on metabolic health. Lipid droplet volume decreased in response to training in oxidative type 1 skeletal muscle. There was an effect of training leading to an increase in perilipin 5 expression that was most prominent in T2DM group.

Lipid droplet-associated Perilipin 2 did not change in response to training in T2DM individuals but was significantly reduced in normal glucose tolerance individuals. While Perilipin 2 and Perilipin 5 are two prominent proteins involved in lipid droplet regulation, they may serve different roles in the management of whole-body glucose regulation and skeletal muscle lipid morphology.

THE EFFECTS OF AEROBIC EXERCISE TRAINING AND INSULIN RESISTANCE ON LIPID DROPLET SIZE AND PERILIPIN PROTEIN ASSOCIATION

by

Evan Ryan Bota

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Arts
2026

Advisory Committee:
Associate Professor Steven J. Prior, Ph.D., Chair
Assistant Professor Sarah S. Glancy, Ph.D.
Associate Professor Sushant Ranadive, Ph.D.

© Copyright by
Evan Ryan Bota
2026

Table of Contents

Table of Contents ii
List of Tables iii
List of Figures iv
Chapter 1: Introduction 1
Chapter 2: Literature Review 4
  Skeletal Muscle Regulation of Glucose Metabolism 4
  Inactivity, Obesity and Insulin Resistance 4
  Characteristics of Intramyocellular Lipids and Lipid Coating Proteins 6
    Formation of Lipid Droplets 6
    Role of Perilipin Proteins 7
    Lipid Droplet Size and Metabolism 9
  Effects of Chronic Aerobic Exercise on Lipid Droplet Morphology 10
    Effects of Aerobic Exercise on Insulin Sensitivity 10
Chapter 3: Rationale and Aims 15
Chapter 4: Methods 19
  Experimental Design 19
    Participant Characteristics and Group Criteria 19
    Aerobic Exercise Training Intervention 19
    Sample Collection 20
  Sample Preparation 22
    Total Perilipin Protein Western Blot 22
    Fluorescent Histology Sample Preparation 23
  Statistical Analysis 27
    Total Perilipin Protein Analysis 28
Chapter 5: Results 29
  Participant Characteristics 29
  Perilipin Protein Expression 30
    Perilipin 2 Expression 30
    Perilipin 5 Expression 31
  Skeletal Muscle Lipid and PLIN Histology 32
    Lipid Droplet Volume 32
    Perilipin 2 and Lipid Droplet Morphology 34
    Perilipin 5 and Lipid Droplet Morphology 36
Chapter 6: Discussion 39
  Skeletal Muscle PLIN Protein Expression 39
  Lipid Droplet Morphology and PLIN Protein Expression 41
  Limitations 44
  Future Directions 45
  Conclusions 46
Kinesiology Research and the School of Public Health 48
Bibliography 52

List of Tables

Table 1. Paired Primary and Secondary Antibody Selections with Concentrations

Table 2. Participant demographics at baseline and post training

Table 3. Lipid Droplet Volume Distribution by Size in Type 1 Fibers

List of Figures

Figure 1. Lipid droplet morphology and perils-in expression in NGT and T2DM, and the effects of aerobic training in T2DM.

Figure 2. Study workflow and experimental design with participant groupings for AEX training intervention.

Figure 3. Skeletal muscle cross-section individual channel and combined.

Figure 4. Relative PLIN protein expression in response to training by glucose tolerance group.

Figure 5. LD-associated PLIN2 by LD Size Group in Response to Training Across Metabolic Groups.

Figure 6. LD-associated PLIN5 by LD Size Group in Response to Training Across Metabolic Groups.

List of Abbreviations

- T2DM – Type 2 Diabetes Mellitus
- LD – Lipid Droplet
- PLIN – Perilipin
- PLIN2 – Perilipin 2
- PLIN5 – Perilipin 5
- TAG – Triacylglycerol
- FA – Fatty Acid
- SS – Subsarcolemmal
- IMF – Intramyofibrillar
- OGTT – Oral Glucose Tolerance Test
- GLUT4 – Glucose Transporter Type 4
- IMTG – Intramuscular Triglycerides
- IMCL – Intramyocellular Lipids
- AEX – Aerobic Exercise Training

Chapter 1: Introduction

The Centers for Disease Control and Prevention (2024), estimate that one in ten Americans is living with diabetes (CDC, 2024); however, lifestyle modifications such as exercise training have been well-established as therapies for insulin resistance by improving skeletal muscle insulin sensitivity and glucose disposal (Merz & Thurmond, 2020). In healthy individuals, as plasma glucose levels rise, insulin is secreted from the pancreas into circulation where it can bind to insulin receptors and subsequently cause glucose uptake. During the progression of insulin resistance, insulin-stimulated glucose uptake is significantly reduced in skeletal muscle, and plasma glucose levels remain elevated (Stanford & Goodyear, 2014). This insulin resistance is, in part, attributable to excess lipid storage, as lipid accumulation has been linked to lipid toxicity in muscle (Bergman & Goodpaster, 2020). Lipid accumulation further disrupts glucose uptake by altering the response of skeletal muscle to insulin. As circulating glucose remains elevated, additional insulin is secreted by the pancreas in an attempt to clear remaining glucose. However, muscle can still take up glucose through exercise-stimulated pathways without insulin, and this mechanism is preserved in type 2 diabetes mellitus (T2DM).

At the muscular level, insulin resistance has been closely associated with lipid droplet (LD) morphology, as larger droplets occur with the lipid toxicity leading to a disruption of the insulin signaling cascade (Gemmink et al., 2017). Lipid droplet proteins, like perilipin 2 (PLIN2), regulate various aspects of lipid morphology. PLIN2 is located on the surface of lipid droplets and stores neutral lipids for future energy demand (Sztalryd & Brasaemle, 2017). Greater total PLIN2 protein expression in muscle has been associated with conditions like sarcopenia, while PLIN2 on lipid droplets has been associated with the formation of larger lipid droplets (Conte et

al., 2013). This is due to PLIN2 inhibiting the breaking down of triacylglycerols (TAG) to free fatty acids. The presence of PLIN2 on larger lipid droplets contributes to the reduction in lipid turnover and increased accumulation of lipid metabolites that block the insulin signaling cascade, preventing skeletal muscle glucose uptake (Sztalryd & Brasaemle, 2017).

While PLIN2 expression on lipid droplets promotes fatty acid uptake, different surface proteins are required to liberate and utilize stored lipids. Perilipin 5 (PLIN5) is a droplet surface protein that aids in the transport of fatty acids from lipid droplets to mitochondria for beta oxidation. When PLIN5 is expressed on the lipid droplet surface, it acts as a tethering mechanism between the droplet and the mitochondrial membrane to facilitate fatty acid transfer (Gemmink et al., 2021).

The balance between lipid storage and utilization is maintained when PLIN5 expression is localized between the lipid droplet and mitochondria, while PLIN2 is non-competitively expressed around the remaining perimeter of the droplet. Total skeletal muscle PLIN5 protein expression has shown to increase with aerobic exercise (AEX) training, suggesting potential enhancements in lipid utilization with physical activity. It must also be noted that PLIN5 is not solely expressed on the lipid droplet surface and can also be found unbound in the cytoplasm. This distinction and importance of the localization of PLIN5 represents a current gap in knowledge and warrants additional research on the expression of PLIN5 on the lipid surface since total protein content is typically reported instead of protein expression by lipid droplet (Parry & Glancy, 2021). Moreover, overexpression of PLIN5 may contribute to lipotoxic accumulation when free fatty acid transport exceeds the capacity to utilize fatty acids (Harris et al., 2015). In insulin resistance, a reduction in PLIN5 expression on lipid droplets may also dampen lipid utilization, leading to greater lipid droplet diameters. These findings highlight the

importance of PLIN protein normalization to individual lipid droplets instead of relying on total PLIN5 expression from the muscle sample, which is a gap in current literature.

In addition to greater lipid utilization, exercise training enhances skeletal muscle mitochondrial content and oxidative capacity. This adaptation is important to consider while assessing differences in skeletal muscle lipid droplet diameter since improved mitochondrial content may also contribute to intramyocellular lipid morphology. By accounting for mitochondrial differences, the effects of exercise training on insulin sensitivity and skeletal muscle lipid morphology can be better understood. Increased mitochondrial content improves the potential for PLIN5 to tether lipid droplets to the mitochondrial membrane for fatty acid transfer. As oxidative capacity improves with exercise training, average lipid droplet diameter may decrease, shifting PLIN2 expression toward lipid neutralization of toxic intermediates over preservation of larger lipid droplets for future metabolic demand.

The relationship between intramyocellular lipids, perilipin proteins and their contributions to glucose homeostasis remains unclear in T2DM, as does the response of these proteins to exercise interventions. Recent literature suggests that determining PLIN localization to lipid droplets and expressing PLIN2 and PLIN5 relative to lipid droplet size may be more relevant to insulin sensitivity and glucose regulation (Gemmink et al., 2021). Therefore, the aim of this thesis is to determine perilipin expression and localization using western blotting and immunohistochemical analyses in order to determine any differences in across glucose tolerance groups with similar physical activity history, then to determine the effects of aerobic exercise training on perilipin-lipid droplet morphology across glucose tolerance groups.

Chapter 2: Literature Review

Skeletal Muscle Regulation of Glucose Metabolism

Skeletal muscle utilizes both carbohydrates and fats as fuel, and the primary fuel source is dependent on factors like fed state and current level of physical activity (Richter et al., 2025). After meal consumption, carbohydrates enter the bloodstream as glucose, which elevates circulating blood glucose levels and promotes pancreatic β-cell insulin production and secretion into the bloodstream (Evans et al., 2019). Circulating insulin diffuses to the interstitial space and binds to insulin receptors on the plasma membrane of skeletal muscle, initiating a signaling cascade to stimulate glucose uptake and clearance from circulation (Yunn et al., 2023). Healthy skeletal muscle is the primary site for insulin-stimulated and meal-dependent glucose disposal, accounting for 70-90% of glucose clearance from the bloodstream (Baron et al., 1988; DeFronzo et al., 1981; Evans et al., 2019). As blood glucose levels decrease, the pancreas reduces insulin secretion, and plasma levels return to their basal levels. Skeletal muscle glucose uptake can also happen through exercise-stimulated pathways that do not require insulin signaling (Lund et al., 1995). In both insulin-stimulated and exercise-stimulated pathways, glucose transporter type 4 (GLUT4) proteins translocate from storage vesicles to the skeletal muscle membrane for glucose uptake. Once glucose enters resting skeletal muscle, it is synthesized into glycogen and stored within muscle for future energy demands (Whytock & Goodpaster, 2025).

Inactivity, Obesity and Insulin Resistance

Diabetes Mellitus (T2DM) is a metabolic disease where individuals live with chronically elevated blood glucose levels, or hyperglycemia. In T2DM, hyperglycemia can be caused by reduced insulin secretion, reduced insulin receptor sensitivity, or a combination of both; but type 2 diabetes mellitus (T2DM) typically begins with insulin resistance (American Diabetes

Association, 2012). During early insulin resistance, circulating glucose levels remain elevated, and the pancreas continues signaling for insulin secretion until blood glucose returns to basal levels (DeFronzo & Tripathy, 2009). Over time, insulin secretion may decrease or even stop completely as the pancreas can no longer supply insulin and insulin receptors no longer respond to insulin-stimulated glucose uptake.

The development of insulin resistance is due to a variety of factors including physical inactivity, poor nutrition and genetic susceptibility (Bellou et al., 2018; Stanford & Goodyear, 2014). Typically, resistance to insulin builds with the accumulation of risk factors like consuming large amounts of processed meat and beverages with high sugar content (Bellou et al., 2018). Diets high in fat and sugar can increase pro-inflammatory molecules in many tissue types and also lead to the development of chronic low-grade inflammation (Ma et al., 2022). With chronic inflammation, the pro-inflammatory cytokine tumor necrosis factor α (TNF-α) can diminish skeletal muscle insulin sensitivity by altering the phosphorylation of IRS-1 (Aguirre et al., 2000). TNF-α activates the cell stress response, which can independently cause serine phosphorylation of IRS-1, and inhibits tyrosine phosphorylation of IRS-1 (Aguirre et al., 2002; Hilder et al., 2003). Tyrosine phosphorylation is a crucial step in skeletal muscle glucose uptake, and altered phosphorylation shifts downstream signaling effects. Since glucose uptake is inhibited by serine phosphorylation of IRS-1, skeletal muscle must rely on fats to match energy demands.

Following a lifestyle of poor diet and chronic stress heightens the risk of obesity. Obese individuals typically store excess fat in their abdominal region and have greater fasting free fatty acids compared to non-obese individuals (Arner & Rydén, 2015). These free fatty acids are transported into skeletal muscle and are stored for future energy demands. Due to serine

phosphorylation of IRS-1, skeletal muscle must rely on oxidative metabolism to produce energy, but mitochondrial content and oxidative capacity are highly influenced by physical activity status. Sedentary individuals typically have lower mitochondrial content, but adaptations can fluctuate based on training history and current physical activity status (Phielix et al., 2010). As a result, T2DM individuals with elevated circulating free fatty acids and low mitochondrial content uptake fatty acids at a rate exceeding skeletal muscle oxidation. This leads to lipid accumulation, and the production lipid intermediates from incomplete utilization.

Characteristics of Intramyocellular Lipids and Lipid Coating Proteins

Formation of Lipid Droplets

Fatty acids are transported from plasma into skeletal muscle through fatty acid transport proteins which are upregulated in response to insulin and skeletal muscle contraction (Kazantzis & Stahl, 2012; Morales et al., 2017). Once fatty acids are transported into skeletal muscle, they can be synthesized as triacylglycerol (TAG) or immediately transported to the mitochondria for oxidation (Kanaley et al., 2009). In the absence of immediate energy demand, skeletal muscle typically stores fatty acids in the lipid neutral form of TAGs, but this is dependent on the current metabolic state of skeletal muscle (Kanaley et al., 2009; Morales et al., 2017).To increase skeletal muscle lipid content, fatty acid uptake must exceed the current rate of fatty acid oxidation.

At rest, intramyocellular lipids (IMCL) are primarily stored in LDs, which act as fuel storage vesicles. The core of each LD consists of neutral lipids, like TAGs, and are surrounded by a phospholipid monolayer (Walther & Farese, 2012). Proteins on the LD monolayer regulate lipid droplet dynamics including lipid storage, transport, and tethering to muscle components like

mitochondria for oxidation. In early studies, it was suggested that insulin resistance and lipotoxicity were driven by the total accumulation of lipids, including TAGs (Jacob et al., 1999). However, this concept did not hold true while accounting for sex differences, as women have a greater skeletal muscle lipid content without impairments to insulin sensitivity (Høeg et al., 2009). Additionally, total muscle lipid content is similar between insulin sensitive endurance athletes and T2DM (Goodpaster et al., 2001). One notable difference explaining similarities in lipid content without insulin resistance in endurance athletes is greater mitochondrial content. While athletes typically have greater mitochondrial content, differences through transmission electron microscopy and citrate synthase activity report conflicting results (Genders et al., 2020). Greater mitochondrial content may provide a greater opportunity for lipid uptake, but lipids must be in proximity of the mitochondria to be oxidized.

Role of Perilipin Proteins

Perilipin (PLIN) proteins are a family of five lipid droplet proteins involved in the regulation of lipid storage and utilization. PLINs can either be exclusively stable on the surface of LD or expressed on both LD surface and in the cytoplasm (Sztalryd & Brasaemle, 2017). In adipose tissue, PLINs are the primary proteins involved in TAG lipolysis, but their involvement in muscle varies by protein. Among PLIN proteins, PLIN2 and PLIN5 proteins are the most highly expressed LD proteins in skeletal muscle. PLIN2 is mostly expressed on the LD surface while PLIN5 is believed to be stable on the LD surface and in the cytoplasm (Gemmink et al., 2017, 2021). To better understand the role of PLINs in skeletal muscle, PLIN2 knockout models were created with C2C12 myotubes. In this knockout model, muscle cells transformed fatty acids into neutral lipids and TAGs at a slower rate, indicating the involvement of PLIN2 in fatty acid storage (Bosma et al., 2012). Similarly, rodent models with PLIN5 knockout had no difference in

total TAG content, but oxidative muscle fibers had less TAG content compared to normally expressing PLIN5 mice (Mason, Mokhtar, et al., 2014). This led to greater TAG content in more glycolytic muscle fibers, indicating the involvement of PLIN5 in oxidative processes including lipolysis. Mice also demonstrated greater respiratory exchange ratios, indicating a greater reliance on carbohydrate over fat metabolism. Interestingly, the greater reliance on carbohydrates were accompanied by lower rates of glucose disposal and muscle glycogen content (Mason, Mokhtar, et al., 2014). This suggests that in the absence of PLIN5, skeletal muscle glucose uptake is lower even as the mice had a greater reliance on glucose during 24-hour energy demands, which could potentially lead to muscle breakdown.

Between studies assessing the role of PLIN proteins in skeletal muscle, there is high variability in reporting measures. This variability has led to conflicting conclusions and results seeming contradictory until methods are compared between studies. Within the current literature, PLIN5 has been noted as dispensable in skeletal muscle by one study, while another describes PLIN5 as a protective protein from lipotoxicity (Laurens et al., 2016; Mohktar et al., 2016). As previously mentioned, PLIN5 is expressed on the LD phospholipid monolayer and the cytoplasm. Immunofluorescent imaging of human muscle biopsies found that only 55% of PLIN5 is colocalized to LDs during rest and exercise (Mason, Meex, et al., 2014). This illustrates that only a fraction of total PLIN5 protein is actively assisting in LD utilization. The earlier mentioned study describing PLIN5 as dispensable utilized a PLIN5 knockout mouse model that effectively reduced mRNA expression by ~70% in skeletal muscle. While understanding only a fraction of PLIN5 is expressed on the LD surface, it is highly plausible the remaining 30% still contributed to lipolysis in the knockout model. This highlights the importance of reporting

alterations in protein expression through a combination of visualization and quantification since total PLIN5 expression may not accurately depict expression on the LD.

Lipid Droplet Size and Metabolism

Similarly to total PLIN protein content, total lipid content alone does not accurately depict LD morphology in the context of insulin resistance. Generally, as total lipid content increases in skeletal muscle, insulin resistance also increases (Jacob et al., 1999). This correlation does not hold under all conditions though as the athlete's paradox identifies similarities in total lipid content between insulin sensitive athletes and sedentary individuals with insulin resistance (Goodpaster et al., 2001). Microscopy analyses reveal T2DM muscle distributes lipids differently throughout the muscle compared to insulin sensitive controls and athletes.

In T2DM, over 40% of lipid contribution in skeletal muscle can be attributed to subsarcolemmal (SS) LD formation (Nielsen et al., 2009, 2017). The accumulation of LDs in the SS region may lead to greater disruptions between insulin signaling proteins compared to LDs found in the intramyofibrillar (IMF) region. In response to training, individuals with T2DM displayed less SS lipid volume without changes in IMF lipid volume, while also displaying greater rates of insulin-stimulated glucose disposal (Nielsen et al., 2009). The location of a LD likely contributes to the type of cellular activity it will be utilized for (Seibert et al., 2020). A droplet located in the SS region may have a greater contribution to membrane activity, while IMF droplets provide energy for skeletal muscle contraction (Hood, 2001; Seibert et al., 2020). Changes in LD diameter can be found after completing a single moderate intensity exercise session, as skeletal muscle present with a greater number of small LDs and fewer large droplets

(Bajpeyi et al., 2023). This may be attributed to large LD utilization and FA neutralization to form smaller droplets.

As lipids are utilized, it would be expected that total PLIN2 expression would decrease, but this would not account for changes in surface area to volume differences. As a single LD volume decreases, a greater percentage of the LD is now on the perimeter. Without differences in total lipid volume, PLIN2 content would now increase to account for the greater amount of LD surface area throughout the muscle. So, PLIN2 content can increase without altering glucose regulation in response to exercise; due to the greater proportion of LD surface area available compared to total lipid volume in skeletal muscle (Shaw et al., 2012). In T2DM, PLIN2 protein is more abundant on larger LDs compared to exercise trained muscle, which may indicate larger lipid droplets are being inhibited from effective utilization (Gemmink et al., 2021). To utilize fatty acids, PLIN5 must be located between the LD and mitochondria. If PLIN2 and PLIN5 are competitively expressed between the LD and mitochondria, fatty acid uptake may be disrupted (Gemmink et al., 2018). Together, these findings highlight the importance of microscopy to identify differences in PLIN protein expression in relation to LDs, as total protein content alone may not accurately represent subcellular differences.

Effects of Chronic Aerobic Exercise on Lipid Droplet Morphology

Effects of Aerobic Exercise on Insulin Sensitivity

Exercise has shown to improve a variety of factors linked to glucose regulation and insulin resistance. Both AEX and resistance training improve glucose regulation in T2DM, but AEX demonstrates greater benefits in total body fat reduction and lipid metabolism (Yang et al., 2014; Zhao et al., 2025). Following 8-months of AEX, fasting insulin can be significantly

reduced, while no improvements are found in fasting glucose or fasting insulin with resistance training (AbouAssi et al., 2015). Training improvements are also noted in the postprandial response to insulin. During a 2-hour oral glucose tolerance test (OGTT), insulin area under the curve was reduced after training without changes in insulin release index (Nassis et al., 2005). In this context, insulin release index during the OGTT was measured as the difference from fasting circulating insulin to 30 minutes into the OGTT divided by the difference from fasting glucose to the same 30-minute timepoint.

While lowering fasting insulin is one important factor in glucose regulation, skeletal muscle insulin sensitivity must increase to adequately dispose glucose. With exercise, improved glucose clearance rates can be found after six weeks of training, but this was in response to a high dose of insulin (Shojaee-Moradie et al., 2007). Longer study durations or increasing training session times may be required to elicit similar results to a low insulin dose response. During insulin-stimulated skeletal muscle glucose uptake, tyrosine phosphorylation of IRS-1 occurs, leading to the translocation of GLUT4 and skeletal muscle glucose uptake (Knudsen et al., 2020). IRS-1 can also be serine phosphorylated, which inhibits the insulin-stimulated glucose uptake pathway (Hilder et al., 2003; Wu & Ballantyne, 2020; Yunn et al., 2023). Thus, total IRS-1 may not be an accurately predict skeletal muscle insulin sensitivity, as it does not account for the phosphorylation or the type of phosphorylation (Jorge et al., 2011).

Alternative pathways that rely on exercise can stimulate skeletal muscle glucose uptake, even when muscle becomes resistant to insulin. During moderate intensity aerobic exercise in T2DM, blood glucose levels decline as peripheral tissues utilize glucose at a rate greater than glucose production (Martin et al., 1995). This was supported by an earlier finding that effectively removed the action of insulin in rats before perfusing and electrically stimulating the hindlimbs.

With the removal insulin, skeletal muscle glucose uptake was greater in contracted muscle compared to the muscle at rest (Ploug et al., 1984). Both insulin stimulation and exercise stimulation result in the translocation of GLUT4, but the signaling pathways are vastly different and are better understood by assessing variations in muscle contraction types (Lund et al., 1995). During eccentric muscle contractions, skeletal muscle is lengthened while actively contracting in the opposing direction. Resistance training emphasizes this type of contraction as it results in greater muscle damage and inflammation, but this inflammatory mechanism also blunts the rate of glucose disposal while performing downhill running (Kirwan et al., 1992). Thus, it was revealed that exercise stimulated glucose uptake is a combination of molecular signaling leading to GLUT4 translocation and nitric oxide dependent vasodilation coupled with enhanced microvascular recruitment (Cacicedo et al., 2011). Previously exercised muscle can retain the effects of exercise stimulated insulin sensitization for up to 48 hours following the last bout of exercise (Mikines et al., 1988).

In response to exercise training, skeletal muscle microvascular networks improve substrate delivery as an adaptation for future requirements. Aerobic exercise (AEX) training improves microvascular function as inactive muscle is recruited during activity. In sedentary obese individuals, improvements in capillarization can be found after six to twelve weeks training (Whytock & Goodpaster, 2025). After six months of AEX training and weight loss interventions, older adults show improvements in capillary density that was also related to glucose clearance and insulin sensitivity (Prior et al., 2014). Additionally, training adaptations to insulin sensitivity and capillarization are partially retained following a period of detraining, demonstrating the importance of microvascular networks in glucose disposal (Prior et al., 2015).

Enhanced capillary networks expand avenues for skeletal muscle glucose clearance and enhance skeletal muscle substrate availability for mitochondrial beta oxidation.

With AEX training, skeletal muscle can express greater oxidative potential through increases in the phenotypic expression of oxidative fibers, and by improving mitochondrial oxidative capacity (Lee & Song, 2018; Tøien et al., 2023). In sedentary individuals, citrate synthase activity, a marker of mitochondrial content and cellular respiration, improved by 46% after 16-weeks of AEX training (Short et al., 2003). Interestingly, improvements in citrate synthase activity were accompanied by improvements in triglycerides and insulin sensitivity index but not fasting insulin or glucose measures. This aligns with a more recent position that reduced skeletal muscle oxidative capacity contributes to insulin resistance due to the accumulation of lipids without effective utilization (Coen et al., 2013). By expanding phenotypic oxidative muscle fiber expression in T2DM muscle, LDs have a greater potential for utilization as oxidative capacity greater than glycolytic muscle fibers (Fachada et al., 2022). Type-II muscle fibers, or more glycolytic muscle fibers, are the ideal low oxygen environment for LDs to accumulate as fatty acid uptake outpaces oxidation.

Conclusions

Overall, current research on insulin sensitivity highlights lipid droplet morphology and PLIN association as informative markers in T2DM pathology. These markers represent a wider range of populations as discrepancies in total lipid content alone can be influenced by aerobic capacity. In individuals with T2DM, enlarged LDs with greater PLIN2 expression have been linked to insulin resistance. Greater PLIN5 protein content may improve lipid oxidation, but the protein must be bound between the LD and mitochondria to facilitate transport. When PLIN5 is non-competitively bound between the LD and mitochondria, lipids turnover at a greater rate and

oxidative metabolism is supported. This underscores the need to evaluate LD size-specific PLIN expression rather than assessing total protein content while addressing skeletal muscle insulin sensitivity.

AEX training can be a beneficial intervention to effectively remodel LDs and aid in the redistribution of PLIN proteins, while also improving oxidative capacity in skeletal muscle. Chronic AEX training alone may not alter total lipid volume, but it can reduce average LD size creating multiple smaller droplets. With improvements in oxidative capacity, PLIN2 expression can favor neutralization of fatty acids as larger LDs are effectively utilized. Together, these training adaptations could enhance skeletal muscle insulin sensitivity, especially in the T2DM population. Further research should rely on a combination of total protein and microscopy techniques to clarify inconsistencies driven by methodological differences. This thesis aims to address these discrepancies by evaluating exercise-induced effects on LD morphology, perilipin association and their intersects between insulin sensitive and T2DM skeletal muscle.

Chapter 3: Rationale and Aims

Skeletal muscle plays a critical role in regulating metabolism and glucose homeostasis through the uptake of glucose during insulin stimulation. In conditions like T2DM, the capacity to effectively utilize intramyocellular lipids becomes impaired, leading to greater accumulation of lipotoxic intermediates and decreased glucose tolerance. In response to regular physical activity, skeletal muscle glucose uptake increases through insulin stimulated and non-insulin stimulated pathways, whereas physical inactivity and obesity are two independent risk factors for skeletal muscle insulin resistance. The accumulation of adipose tissue is caused by factors including poor diet and inactivity, which can raise basal circulating free fatty acid levels. These fatty acids are transported into skeletal muscle, to be stored as lipid droplets and to be utilized for future energy demands. Greater lipid accumulation within skeletal muscle has been associated with reduced insulin sensitivity; however, this relationship may be more strongly influenced by the enlargement of lipid droplets rather than total lipid content. This suggests lipid droplet (LD) morphology and lipid protein expression hold metabolic importance in the context of glucose tolerance.

Intramyocellular LDs are vesicles within skeletal muscle storing neutral lipids as a fuel source. Surface proteins surround the vesicle to regulate actions like lipid storage, turnover and preservation. When excess FFAs are present in muscle, PLIN2 is upregulated and binds during the formation of LDs. In the absence of PLIN2, the capacity to store lipids is reduced and toxic lipid intermediates accumulate within the muscle. During intramuscular triglyceride utilization, PLIN5 expression on the lipid droplet surface increases. PLIN5 docks lipid droplets to the mitochondrial membrane where fatty acids can be transferred for oxidation. Differences in perilipin protein expression by lipid droplet size may further explain the pathology behind lipid

metabolism in insulin resistant skeletal muscle. The proposed methods account for variations in protein expression that could otherwise be influenced by differences in LD diameter to total LD volume.

While aerobic training improves glucose regulation and results in smaller lipid droplet diameters, the effect of AEX training on perilipin protein expression by lipid droplet size remains unknown. AEX training improves oxidative capacity in skeletal muscle, but current literature is unclear about its impact on lipid droplet morphology across the glucose tolerance spectrum in skeletal muscle. Additionally, little is known about the reorganization of lipid droplet proteins by lipid droplet size after exercise training.

This thesis will test the hypothesis that untrained glucose intolerant skeletal muscle will have larger lipid droplet volumes, with greater PLIN2 expression and lower PLIN5 expression on the larger lipid droplets. Additionally, this thesis will determine whether six months of AEX training alters lipid droplet volume with lower PLIN2 expression and greater expression of PLIN5 on larger droplets in glucose tolerant and resistant muscle.

The hypotheses will be addressed by the following aims:

Aim 1: Determine the effects of aerobic exercise training on total skeletal muscle perilipin protein expression in previously sedentary older adults with or without glucose tolerance.

A. Quantify PLIN2 and PLIN5 protein expression in skeletal muscle from glucose tolerant and T2DM individuals to determine the association of glucose tolerance status and total perilipin protein abundance in skeletal muscle.
B. Assess changes in total PLIN2 and PLIN5 protein expression after a six-month aerobic training intervention by glucose tolerance status.

Aim 2: Determine the effects of AEX training on lipid droplet morphology and perilipin protein association in glucose tolerant and resistant skeletal muscle.

A. Quantify the volume of PLIN2 and PLIN5 per lipid droplet to assess baseline distribution patterns between glucose-sensitive and glucose-resistant skeletal muscle.
B. To assess if AEX training decreases lipid droplet diameter, with PLIN2 expression favoring smaller lipid droplets and greater PLIN5 expression on larger lipid droplets in glucose intolerant skeletal muscle.

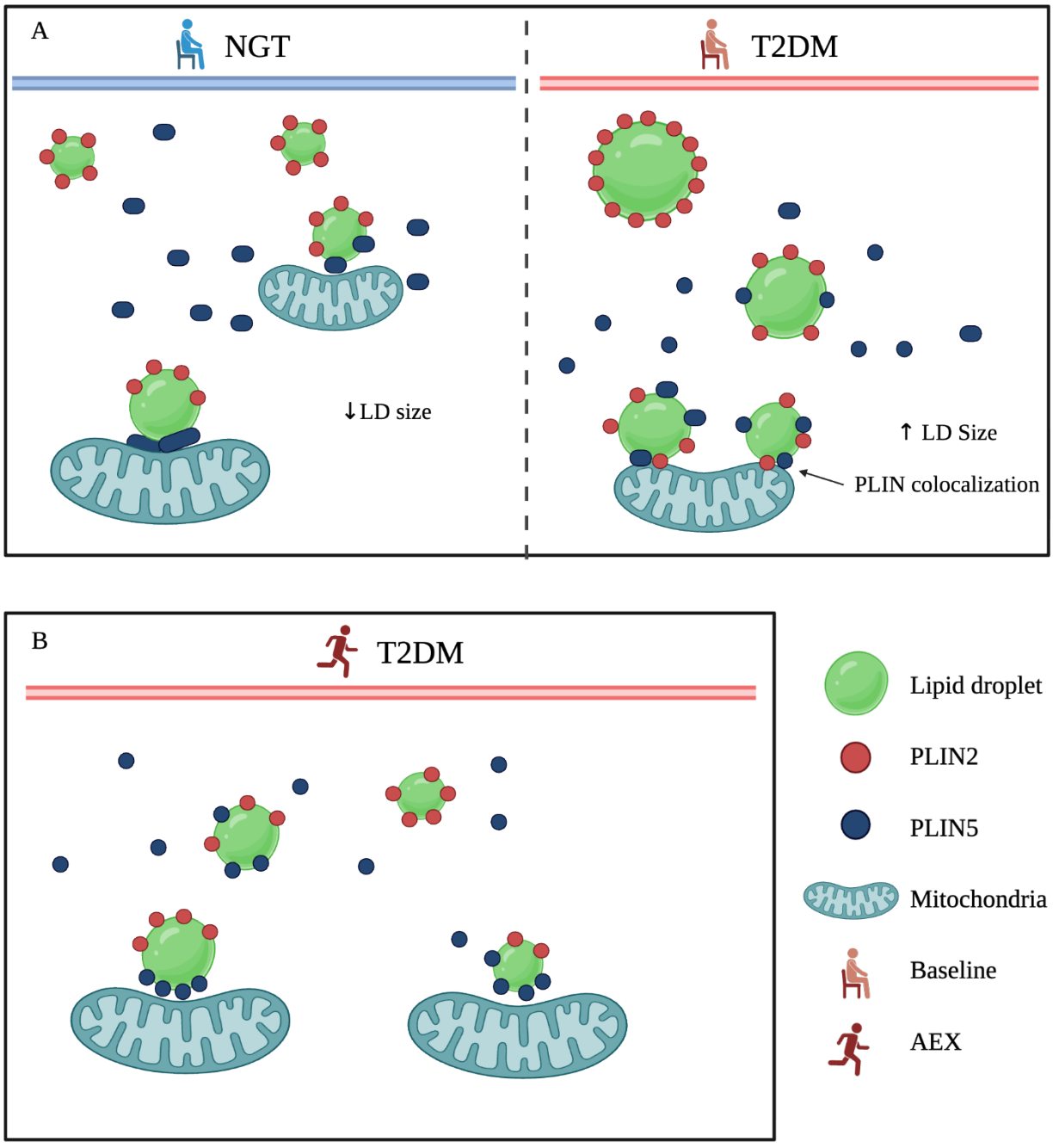


Figure 1. Lipid droplet morphology and perilipin expression in NGT and T2DM, and the effects of aerobic training in T2DM.
(A) Comparison of normal glucose tolerance (NGT) and T2DM skeletal muscle. Individuals with NGT have smaller lipid droplets (LD) with expression of perilipin 2 (PLIN2) favoring small droplets. T2DM muscle have larger LDs with greater expression of PLIN2. Perilipin 5 (PLIN5) is localized between the LD and mitochondria in NGT while PLIN2 and PLIN5 colocalize in T2DM.
(B) Predicted effects of AEX training in T2DM. Muscle will have greater mitochondrial content and reduced LD size. PLIN2 expression on small LDs will be greater while PLIN5 expression will favor large LDs. PLIN5 will express between the mitochondria and LD. These adaptations will enhance lipid turnover.

Chapter 4: Methods

Experimental Design

Participant Characteristics and Group Criteria

To meet eligibility criteria for this study, participants were between the ages of 50-85 years old, body mass index between 18-50 kg/m^2 and women must be postmenopausal for at least one year. All T2DM participants must (1) be on a stable medical regime for at least 30 days, (2) have a Hemoglobin A1c (HbA1c) ≤10% and (3) fasting plasma glucose less than 200 mg/dL. Individuals were excluded from the study if they (1) were previously diagnosed with coronary artery disease, (2) were in active cancer treatment, (3) presented with a blood disease, (4) took oral steroids or medications that could not be safely discontinued for 72 hours, (5) presented with uncontrolled hypertension, (6) had previously been diagnosed with type 1 diabetes, (7) participated in >20 minutes of vigorous exercise >2 days per week or (8) presented with another medical condition determined as criteria for exclusion by the study team prior to participation.

Participants were be classified as T2DM if they had been previously diagnosed by a physician. In the remaining participants, subjects underwent a 2-hour OGTT after a 12 hour overnight fast. A catheter was placed in the antecubital vein with blood samples collected every 30 minutes after consuming a 75g glucose beverage. Participants were classified as having normal glucose tolerance or impaired glucose tolerance based on 2-hour OGTT results.

Aerobic Exercise Training Intervention

During the aerobic exercise intervention, subjects exercised 3x/ week for 6-months at a VAMHCS exercise facility. Exercise intensity was set to 70-80% VO_2max for 30-45 minutes with blood pressure and heart rate assessments before, during and after training sessions.

Subjects were instructed to exercise on their own for 60 minutes during the weekend with their exercise intensity measured using a heart rate monitor. To progress exercise training, subjects started with 30 minutes at 60% VO_2max, then increased training time as tolerated to 45 minutes at 60% VO_2max before increasing exercise intensity to 70% then 80% VO_2max, as tolerated by the participant. Subjects were encouraged to maintain their body weight throughout the intervention with dietary supplementation given when weight loss or gain was greater than 2% per month.

Sample Collection

Subjects were provided with all meals for 2 days prior to sample collection to control for variations in dietary intake. On the day of testing, subjects fasted 12 hours prior to sample collection. Percutaneous needle biopsies were obtained from the vastus lateralis, ~12 cm above the patella using a Bergstrom needle (Stille, Solna, Sweeden). One biopsy sample was immediately embedded in optimal cutting temperature compound and frozen in liquid nitrogen before storage at -80°C for immunofluorescent histology analysis. The remaining samples were immediately freeze-clamped with liquid nitrogen and stored at -80°C for western blotting analysis. Additionally, western blot samples were lyophilized for 48 hours and dissected to be free of connective tissue, fat and vascular cells.

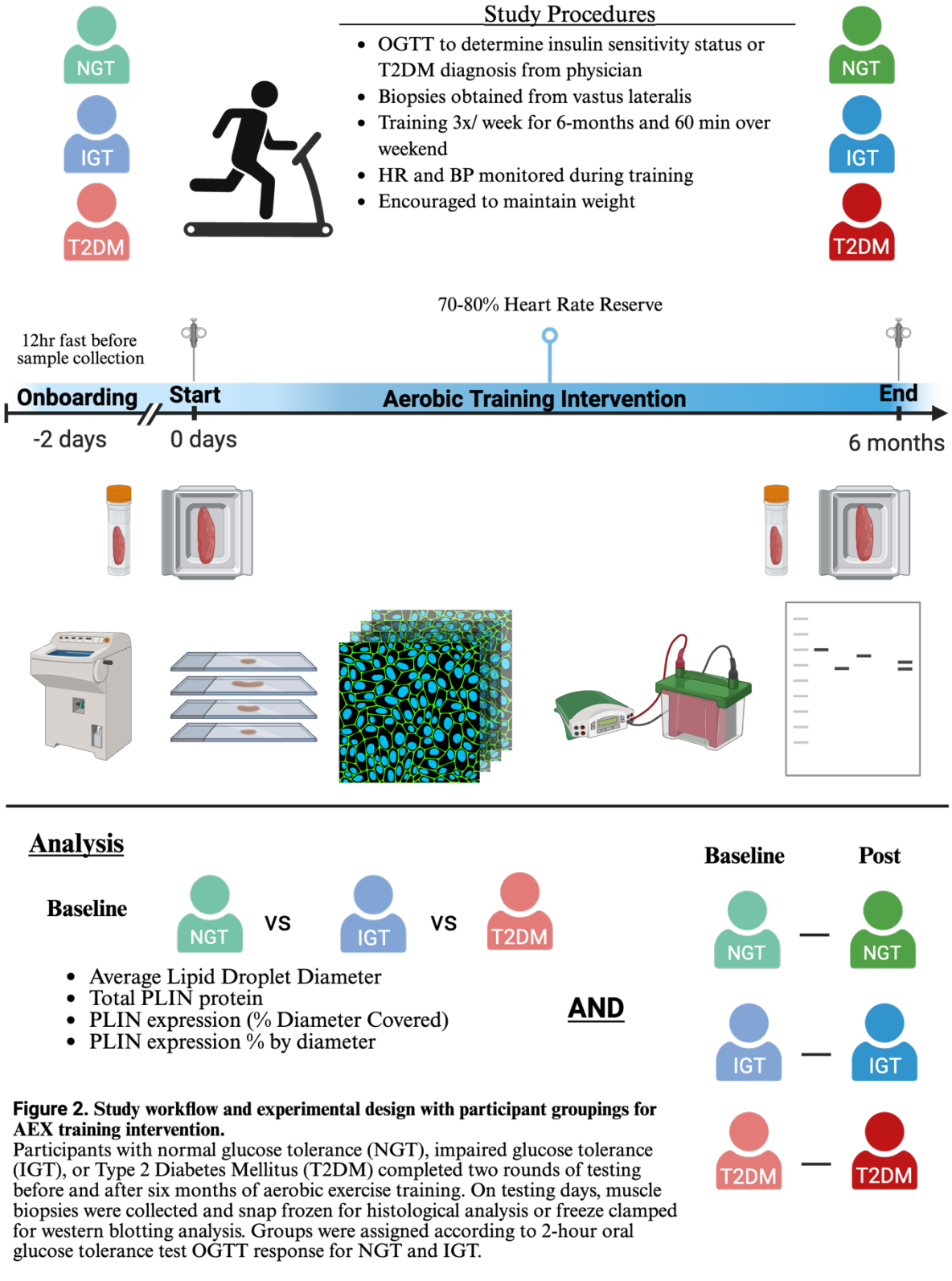


Figure 2. Study workflow and experimental design with participant groupings for AEX training intervention.
Participants with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or Type 2 Diabetes Mellitus (T2DM) completed two rounds of testing before and after six months of aerobic exercise training. On testing days, muscle biopsies were collected and snap frozen for histological analysis or freeze clamped for western blotting analysis. Groups were assigned according to 2-hour oral glucose tolerance test OGTT response for NGT and IGT.

Sample Preparation

Total Perilipin Protein Western Blot

Muscle samples were weighted and homogenized at 10% volume by weight in a triton lysis buffer will containing 0.1% Triton X-100, 1% protease inhibitor and 1% phosphatase inhibitor in deionized water. Samples were centrifuged at 1,000x g for 10 minutes at 4°C and placed back on ice to create aliquots from the collected supernatant. A protein assay (Pierce™ BCA Protein Assay Kit) was performed to calculate total protein concentration for each sample before storage at -80°C in microtubes.

Each western blot lane contained 50 µg of total protein loaded from the previously performed protein assay. Laemmli buffer and beta-mercaptoethanol was prepared [3:1 ratio, respectively] and accounted for one quarter of the total lane volume with the remaining volume being deionized water. Samples were heated for 10 minutes at 65°C and transferred back on ice after heating. Mini-Protean TGX stain free 12 well Gels (Bio-Rad) were used for gel electrophoresis and total protein per well was computed by activating the UV gel and imaging. Protein standards (Bio-Rad) were loaded into a lane in the gel to identify proteins by molecular weight. Samples were then loaded with baseline to post training on the same gel to minimize potential variability between western blots while performing analysis. The gel was imaged for total protein content before wet protein transfer from gel to polyvinylidene difluoride (PVDF) membrane. Protein transfer was performed at a consistent low voltage overnight at 4°C and protein transfer was confirmed by reimaging gel following the same protocol used for total protein quantification.

The PVDF membrane was then blocked with bovine serum albumin (BSA) before washing with Tris-Buffered Saline with Tween-20 (TBST). The membrane was then incubated

for three hours on a rocker at room temperature with primary antibodies against Perilipin 5 [1:2000; Proteintech] in TBST. After washing the membrane, Advin-HRP conjugated secondary antibodies [1:1000; R&D Systems] were added to detect bound primary antibodies for 30 minutes at room temperature. Before imaging, the membrane incubated on a rocker in SuperSignal™ (Thermo Scientific) for 5 minutes at room temperature then directly placed on Chemidoc MP (Bio-Rad) for imaging. Following image acquisition, the membrane was then stripped of antibodies with 1M NaOH for 2 minutes on a rocker before another series of blocking with BSA in TBST for 45 minutes. The membrane then incubated overnight on a rocker at 4°C with primary antibodies against Perilipin 2 [1:2000; Invitrogen] in TBST. The following day, the same process was followed using Advin-HRP conjugated secondary antibodies [1:1000; R&D Systems] and imaging.

To determine PLIN2 and PLIN5 protein abundance, western blot images were analyzed using Bio-Rad Image Lab software (Bio-Rad). Individual lanes on the image were detected, and total protein was measured across the length of each lane. Protein bands for PLIN2 and PLIN5 were confirmed by the molecular weight using the loaded protein standards. Background signal was subtracted from the volume before protein expression normalization. To normalize protein expression, the protein of interest band intensity was divided by the total protein intensity from the images of the activated gel. Data was exported to a spreadsheet for statistical analysis between groups.

Fluorescent Histology Sample Preparation

Biopsy samples embedded in optimal cutting temperature were be used for histological analysis of lipid droplet morphology and perilipin protein association. Samples were be placed in a cryostat set to -21°C for 30 minutes to acclimate prior to sectioning. Sections were cut at 10µm

thickness on glass slides. To limit batch variability, all histological samples were prepared on the same day. Sections were first fixed in Image-iT ™ Fixative paraformaldehyde solution (Invitrogen) to preserve lipid droplets. Slides were then blocked with 5% goat serum in PBS for 45 minutes at room temperature and subsequently treated with 0.25% Triton X-100 in PBS for 5 minutes before washing in PBS. Slides were incubated with primary antibodies against Perilipin 2 [1:500; Invitrogen], Perilipin 5 [1:200; Proteintech] and MYH7- Slow [1:200; DSHB] in 5% goat serum and 0.05% Tween-20 in PBS for 1 hour at room temperature. After washing, slides were then be incubated with fluorescently labeled secondary antibodies [1:500; Invitrogen] in PBS that coordinate with specific target species and antibody isoforms (Table 1). Secondary antibody incubation also contained BODIPY™ 493/503 [1:1000; Invitrogen] (1mg/mL in DSMO) to detect lipid droplets. Slides were incubated for 30 minutes at room temperature in the dark to avoid photobleaching. Sections were then washed in PBS to reduce non-specific binding before applying Prolong Gold mounting medium and number 1.5 coverslip for imaging on the Leica Stellaris 8 Falcon laser scanning confocal microscope.

Table 1. Paired Primary and Secondary Antibody Selections with Concentrations

Application	Primary (Catalog #)	Concentration	Secondary (Catalog #)	Concentration
Western Blot	Perilipin 2 (15294-1-AP)	1:1000	Anti Rabbit HRP Conjugated (HAF008)	1:1000
	Perilipin 5 (26951-1-AP)	1:2000	Anti Mouse HRP Conjugated (HAF018)	1:1000
Histology	Perilipin 2 (15294-1-AP)	1:500	Alexa Fluor 647 (A21240)	1:500
	Perilipin 5 (26951-1-AP)	1:200	Alexa Fluor 405 (A31556)	1:500
	BODIPY 493/503 (D3922)	1:1000	--	--
	MYH7- Slow (A4.840)	1:200	Alexa Fluor 568 (A21043)	1:500

BODIPY image stacks were trained using Ilastik (v1.4.1) object classification to create probability maps of lipid droplets (Berg et al., 2019). Probability maps were then exported for volume measurement using the BoneJ "Particle Analyser" plugin in Fiji (Domander et al., 2021), where both surface resampling and volume resampling were set to one. Regions that expressed MYH7 and BODIPY were counted as type 1 lipid droplets throughout the analysis.

PLIN2 and PLIN5 protein abundance was quantified in ImageJ. Briefly, PLIN images were quantified as both binary images and varying pixel intensity images using the MorphoLibJ Intensity Measurements 2D/3D Fiji plugin (Legland et al., 2016). Relative intensity values were computed by multiplying the total number of PLIN positive pixels by the mean intensity of the PLIN pixels on a singular droplet scale. This measurement returns a relative protein expression calculation that is similar in concept to western blotting quantification, by calculating PLIN expression relative to the droplet it is expressed on.

An extended region approximately twice the spatial resolution was computed around each lipid droplet to capture fluorescent signal surrounding the droplet surface. The number, location and intensity of PLIN positive voxels were given an identification number that was identical to each parent lipid droplet. For each subject and timepoint, lipid and PLIN volume was computed before constructing group statistics.

Confocal z-stack images and lipid droplet probability maps were drawn from 167,800 lipid droplets across samples to assess the effects of insulin sensitivity and training status on lipid droplet morphology. Volume groups are based on conversions from previously reported lipid droplet diameters using the formula: $Volume = \pi * Diameter^3/6$.

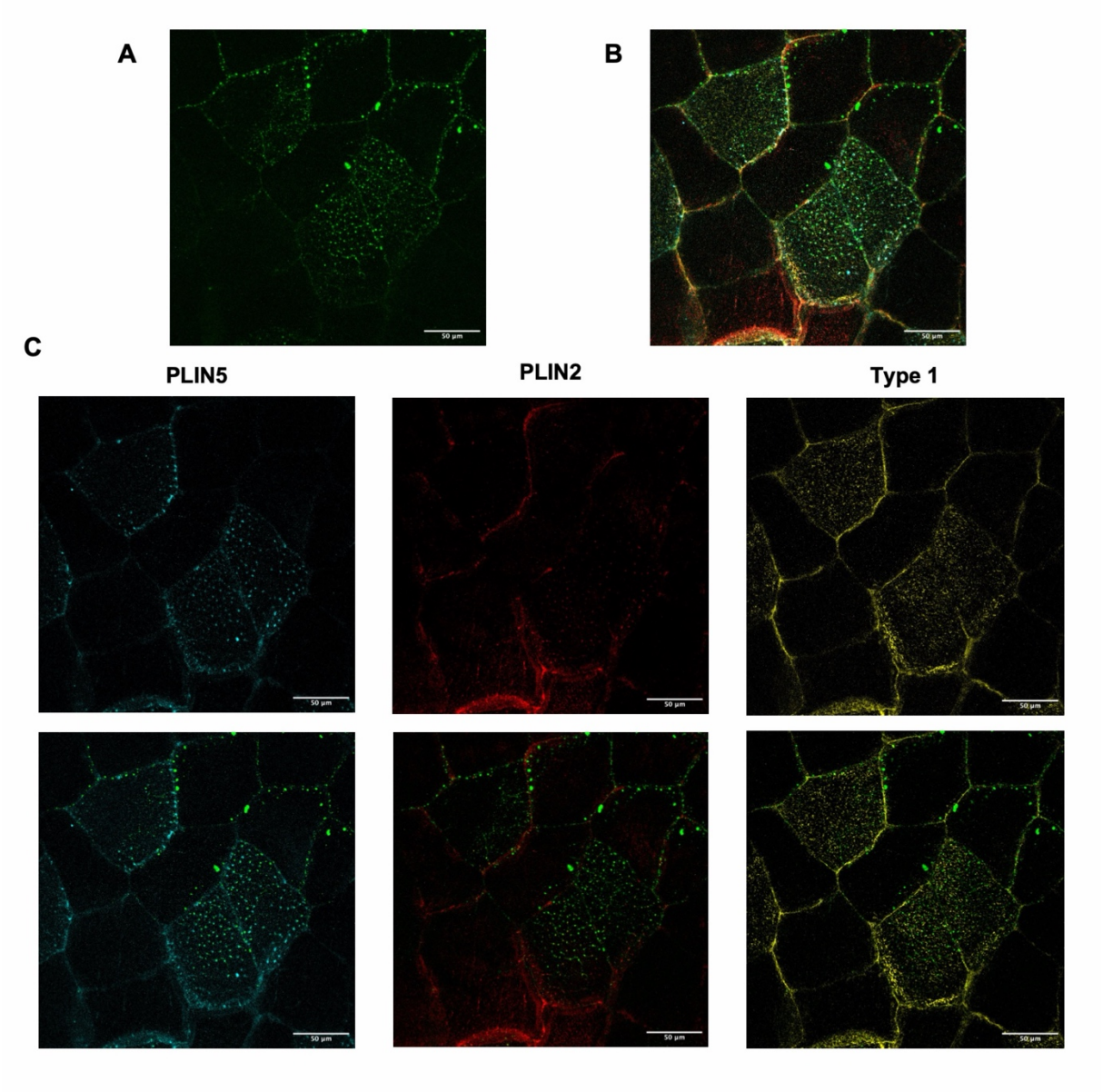


Figure 3. Representative skeletal muscle cross-section of histological components. Fluorescent confocal image of BODIPY 493/503 (A), merged image of all four components (B), and individual channel images of PLIN5, PLIN2 and Type 1 myosin heavy chains with merged BODIPY image below (C). Scale bar is 50µm with images acquired at 2048 × 2048 pixel resolution with spatial resolution of 0.08µm^2.

To assess differences in lipid droplet morphology, the mean lipid droplet volume was computed per subject with a maximum volume set to $3\mu m^3$ to account for skewness of highly variable volumes. The mean droplet size was then averaged across participants within a group (Table 3). Lipid droplet size distributions are presented below the mean volume (Table 3). PLIN protein was quantified as LD-associated PLIN and LD-associated PLIN by LD volume. This was done by identifying all PLIN positive voxels that were also lipid droplet positive voxels. PLIN positive voxels were then converted to cubed microns and multiplied by the mean voxel intensity on per droplet. The LD-associated PLIN measurement provided an absolute expression of PLIN protein on a singular droplet basis that was independent of LD volume. LD-associated PLIN by LD volume was calculated by dividing the previous expression value by the volume of the parent lipid droplet. Both PLIN measures were binned by lipid droplet size group to assess variance in protein expression in an absolute fashion across volume groups and relative to the volume of lipid. Individual lipid droplet protein expression was analyzed using a two-way RM-ANOVA to assess differences by insulin sensitivity group, response to exercise training and lipid droplet size group.

Statistical Analysis

For all analyses, statistical power was set to 80% with an alpha level of 0.05 (two-tailed). An a priori power analysis was performed based on findings from Nielsen et al. (2017) to determine sample size. A minimum sample size of 8 participants will be needed to detect differences in LD size between insulin-sensitive and insulin-resistant groups ($0.55 \pm 0.11\mu m$ and $0.77\mu m$; respectively). Similar sample sizes are also required to detect changes in total PLIN protein expression between groups and timepoints. Based on previously reported western blot

data, a minimum sample size of six is needed to determine differences in PLIN protein expression from skeletal muscle homogenates (Daemen et al., 2018).

Total Perilipin Protein Analysis

Effects of insulin sensitivity group and training on total perilipin protein expression from skeletal muscle homogenates were analyzed using a two-way RM-ANOVA. Comparisons were selected to compare group differences at each timepoint and to detect within group differences in response to training.

Chapter 5: Results

Participant Characteristics

Participants were grouped either by previous diagnosis of T2DM, or by results of an oral glucose tolerance test for the NGT and IGT groups. Participant characteristics are presented in Table 2. There were no differences in weight, lean body mass, fat mass, or body mass index between groups or in response to training. There was an effect of training that caused $\dot{V}O_{2max}$ to increase by 7% across groups. As expected, T2DM fasting glucose was greater than both NGT and IGT at baseline and remained greater after training ($p < 0.001$). At baseline the IGT group had an average 120-minute postprandial glucose level of 158 mg/dL, which was greater than the 99 mg/dL average in the NGT group ($p < 0.0001$). This difference was not mitigated in response to exercise training ($p = 0.008$). Hemoglobin A1c (HbA1c) was not different between NGT and IGT groups at baseline or post training, but the T2DM group reported greater HbA1c values than both groups at each timepoint ($p < 0.05$).

Table 2. Participant demographics at baseline and post training

Variable	Baseline			Post Training		
Glucose Tolerance Groups (Male/Female), n	**NGT** (6/2)	**IGT** (5/6)	**T2DM** (4/3)	**NGT**	**IGT**	**T2DM**
Age	59 ± 9	64 ± 6	62 ± 7			
Height (cm)	173 ± 10	169 ± 12	173 ± 8			
Weight (kg)	87.5 ± 21.1	85.9 ± 16.3	101.7 ± 17.9	87.4 ± 19.9	84.5 ± 18.4	101.0 ± 17.0
BMI (kg/m^2)	28.8 ± 4.7	30.1 ± 5.0	34.3 ± 6.5	28.8 ± 4.3	29.0 ± 4.4	34.0 ± 6.1
Fat mass (kg)	30.3 ± 11.7	34.2 ± 8.9	39.8 ± 15.8	29.8 ± 11.0	31.9 ± 9.3	39.7 ± 15.5
Lean body mass (kg)	52.5 ± 11.1	46.8 ± 9.8	55.2 ± 9.5	52.4 ± 11.4	47.6 ± 9.6	55.3 ± 9.3
Fat mass (%)	33.8 ± 7.7	39.7 ± 6.5	38.8 ± 10.3	33.7 ± 8.2	37.2 ± 5.5	38.6 ± 10.0
VO_{2max} (ml/kg/min)	28.7 ± 7.1	24.0 ± 3.6	22.3 ± 6.1	31.3 ± 9.9$^{!}$	25.2 ± 4.1$^{!}$	24.8 ± 6.3$^{!}$
Fasting glucose (mg/dL)	89 ± 7	102 ± 12	146 ± 34$^{*\#}$	91 ± 8	96 ± 7	148 ± 38$^{*\#}$
120-min Postprandial Glucose (mg/dL)	99 ± 26.0	158 ± 21.5*	N/A	113 ± 29	148 ± 26*	N/A
HbA1c	5.5 ± 0.2	5.6 ± 0.4	7.1 ± 1.3$^{*\#}$	5.5 ± 0.2	5.6 ± 0.3	7.0 ± 0.8$^{*\#}$

Data are means ± standard deviation. NGT Normal Glucose Tolerance, IGT Impaired Glucose Tolerance, T2DM Type 2 Diabetes Mellitus. Only fasting glucose measurement collected from T2DM group. *P-value <0.05 from NGT. $^{\#}$P-values <0.05 from IGT. $^{!}$P-values <0.05 significant main effect of training.

Perilipin Protein Expression

Perilipin 2 Expression

Training increased PLIN2 protein expression in skeletal muscle homogenates regardless of glucose tolerance status. At baseline, PLIN2 protein expression did not differ among NGT, IGT and T2DM groups (Figure 4A, p = 0.41). In response to aerobic exercise training, PLIN2 protein expression increased 25-30% across all groups (main effect p = 0.04). There were no differences between NGT, IGT or T2DM after training (p = 0.44), which suggests glucose intolerant skeletal muscle maintains the capability to upregulate PLIN2 expression in response to exercise.

Perilipin 5 Expression

In response to training, PLIN5 protein expression also increased by 35% across groups (main effect $p = 0.02$, Figure 4B); however, the response to training was the greatest in the T2DM group, which had greater relative protein expression compared to the NGT group at the same timepoint ($p = 0.02$). This was a difference in response to training as between group differences were not present at baseline. The T2DM group was the only group to also demonstrate greater PLIN5 protein abundance from baseline to post training, with average PLIN5 expression increasing upwards of 50% in each participant ($p = 0.03$, Figure 4). The T2DM group increase may indicate that after six months of training T2DM individuals have the greatest potential for continued metabolic improvements. There was no interaction between training and glucose tolerance status ($p = 0.37$), but the variation in post training response may still hold relevance in lipid droplet morphology and the regulation of insulin resistant muscle.

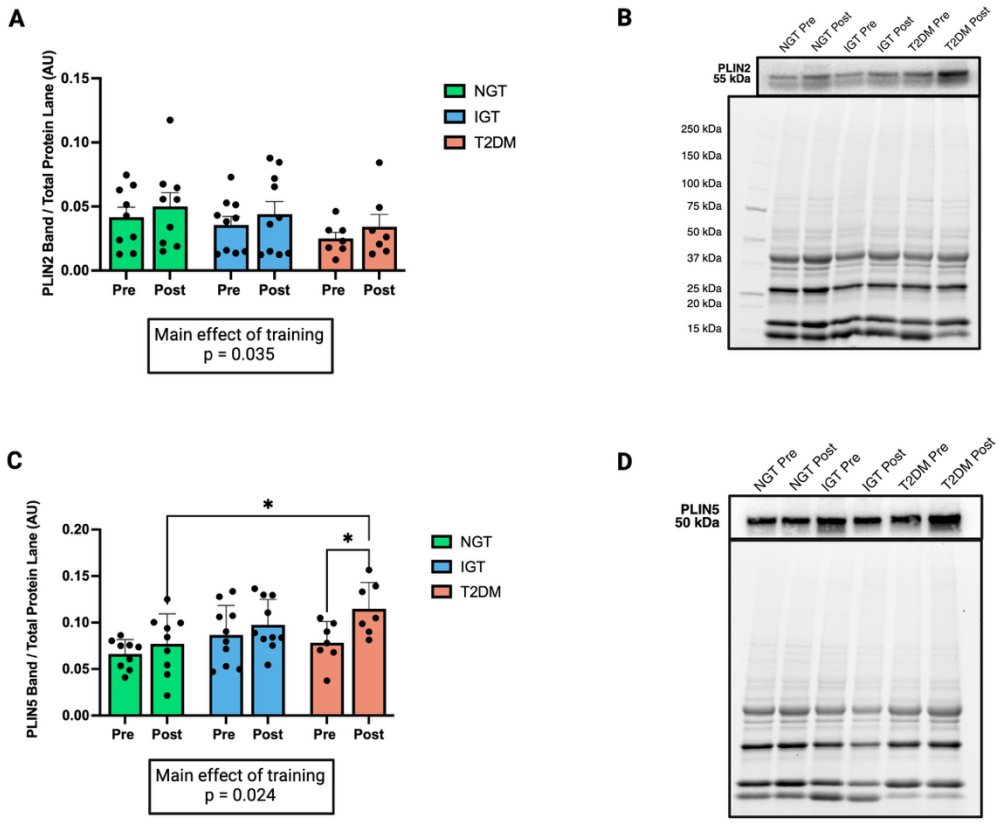


Figure 4. Relative PLIN protein expression in response to training by glucose tolerance group. Relative PLIN2 (A) and PLIN5 (C) expression by glucose tolerance group and training status; (B & D) representative chemiluminescent protein bands and total protein gel images labeled by group and timepoint. Data are mean ± SEM. *P-value < 0.05.

Skeletal Muscle Lipid and PLIN Histology

Lipid Droplet Volume

Lipid droplet volume frequency distributions were compared among nine different size groups across glucose tolerance groups and timepoints. Two size categories, (0-0.05µm^3) and (0.32-0.45µm^3) had significant differences between groups and across timepoints. The smallest LD size group generally was the most prevalent with a range of 17- 41% of total LDs across glucose tolerance groups and training status. At baseline, the IGT group had a greater proportion of lipid droplets than the T2DM group in the smallest size bin (p = 0.02), but there was no

difference between NGT and T2DM groups (p = 0.39). Following training, the IGT group had significantly lower contributions of lipid droplets within the 0-0.05μm^3 size group (Table 3; p = 0.03).

In the larger (0.32-0.45μm^3) volume bin, T2DM individuals presented with ~12% of droplets within the size range, which was significantly greater than both NGT (8.8%, p = 0.04) and IGT (6.7%, p = 0.003) groups. Following training, the T2DM group had a reduced contribution of LDs within the 0.32-0.45μm^3 size bin, going from 12% to 6.8% (p = 0.001). This decline also led to no differences in proportions across groups following training. The response in the T2DM group suggests a shift in LD morphology in oxidative muscle fibers towards smaller LD storage. The 0.32-0.45μm^3 size group correlates to a diameter of 0.85-0.95μm, which has been traditionally noted as large lipid droplets.

Table 3. Lipid Droplet Volume Distribution by Size in Type 1 Fibers

	Baseline			Post Training		
Measurement	**NGT**	**IGT**	**T2DM**	**NGT**	**IGT**	**T2DM**
Individual LD Surface Area (μm^2)	3.7 ± 0.5	2.8 ± 0.63	4.0 ± 0.4	3.1 ± 1.1	4.3 ± 1.7	4.0 ± 0.7
Individual LD Volume (μm^3)	0.66 ± 0.09	0.44 ± 0.15	0.72 ± 0.09	0.54 ± 0.22	0.69 ± 0.28	0.73 ± 0.14
Size Frequency (%)						
0-0.05 μm^3	27.0 ± 5.9	41.2 ± 7.8*	17.9 ± 3.6	33.1 ± 10.5	22.8 ± 12.2!	22.7 ± 9.9
0.06-0.11 μm^3	9.6 ± 1.5	11.6 ± 1.9	9.0 ± 1.5	10.6 ± 1.6	9.0 ± 3.2	9.8 ± 2.2
0.12-0.16 μm^3	5.7 ± 0.4	5.9 ± 1.1	6.2 ± 0.9	6.2 ± 1.1	6.9 ± 0.9	5.8 ± 1.5
0.17-0.31 μm^3	8.4 ± 0.3	7.8 ± 0.9	9.3 ± 1.6	8.6 ± 1.0	9.0 ± 2.8	9.0 ± 1.3
0.32-0.45 μm^3	8.8 ± 0.7*	6.7 ± 1.5*	12.0 ± 2.9	8.3 ± 0.5	9.4 ± 1.0	6.8 ± 1.7!
0.46-0.69 μm^3	4.0 ± 0.5	3.7 ± 0.3	5.1 ± 1.4	4.0 ± 0.4	4.7 ± 1.8	4.5 ± 1.6
0.7-1.39 μm^3	19.3 ± 3.4	12.7 ± 4.0	21.7 ± 2.7	15.9 ± 4.4	18.8 ± 5.4	21.8 ± 4.6
1.4-2.29 μm^3	9.9 ± 1.7	6.2 ± 2.5	11.2 ± 1.5	7.6 ± 4.4	11.3 ± 5.9	10.9 ± 1.7
2.3-3.0 μm^3	7.4 ± 1.5	4.2 ± 2.7	7.6 ± 2.0	5.6 ± 4.3	8.0 ± 6.2	8.7 ± 2.7

Data are mean ± standard deviation in Type 1 muscle. *Significant difference from T2DM group at the same timepoint (p-value <0.05). !Significant within group effect of training (p<0.05).

Perilipin 2 and Lipid Droplet Morphology

To examine LD-associated PLIN2 across different LD sizes, PLIN2 protein that was spatially associated with LDs was quantified within and across the nine LD size categories. Before training, the NGT cohort expressed 32% more LD-associated PLIN2 by LD volume than the IGT group ($p < 0.0001$, Figure 5A) and 20% greater expression compared to the T2DM group ($p = 0.002$). There was no difference between IGT and DM at baseline ($p = 0.054$). This suggests that PLIN2 expression is more densely concentrated surrounding type 1 LDs in NGT muscle, which may be due to their greater ability to switch between lipid and carbohydrate fuel sources. Across groups, larger droplets had more LD-associated PLIN2 ($p < 0.0001$, Figure 5B), which would be expected with volume and surface area increases. This held true regardless of training and was consistent across glucose sensitivity groups.

The response to training on LD-associated PLIN2 by LD volume was not uniform across groups and was dependent on glucose tolerance status. NGT individuals had a 36% reduction in LD-associated PLIN2 by LD volume, and this decline was consistent across LD volume groups ($p < 0.0001$, Figure 5C). This follows the concept that a greater reliance on lipid metabolism occurs as an adaptation to exercise training, and may explain the slight, but insignificant, variation in fasting glucose levels at baseline. In contrast, the IGT cohort had a 17% increase in LD-associated PLIN2 by LD volume following training ($p = 0.033$, Figure 5A), while the T2DM group had no significant change ($p = 0.68$). Interestingly, both glucose intolerant groups expressed 24% more LD-associated PLIN2 by LD volume compared to the NGT group (IGT $p = 0.018$ and T2DM $p = 0.27$). Following training, there was no difference in PLIN2/LD volume between IGT and T2DM groups ($p = 0.97$).

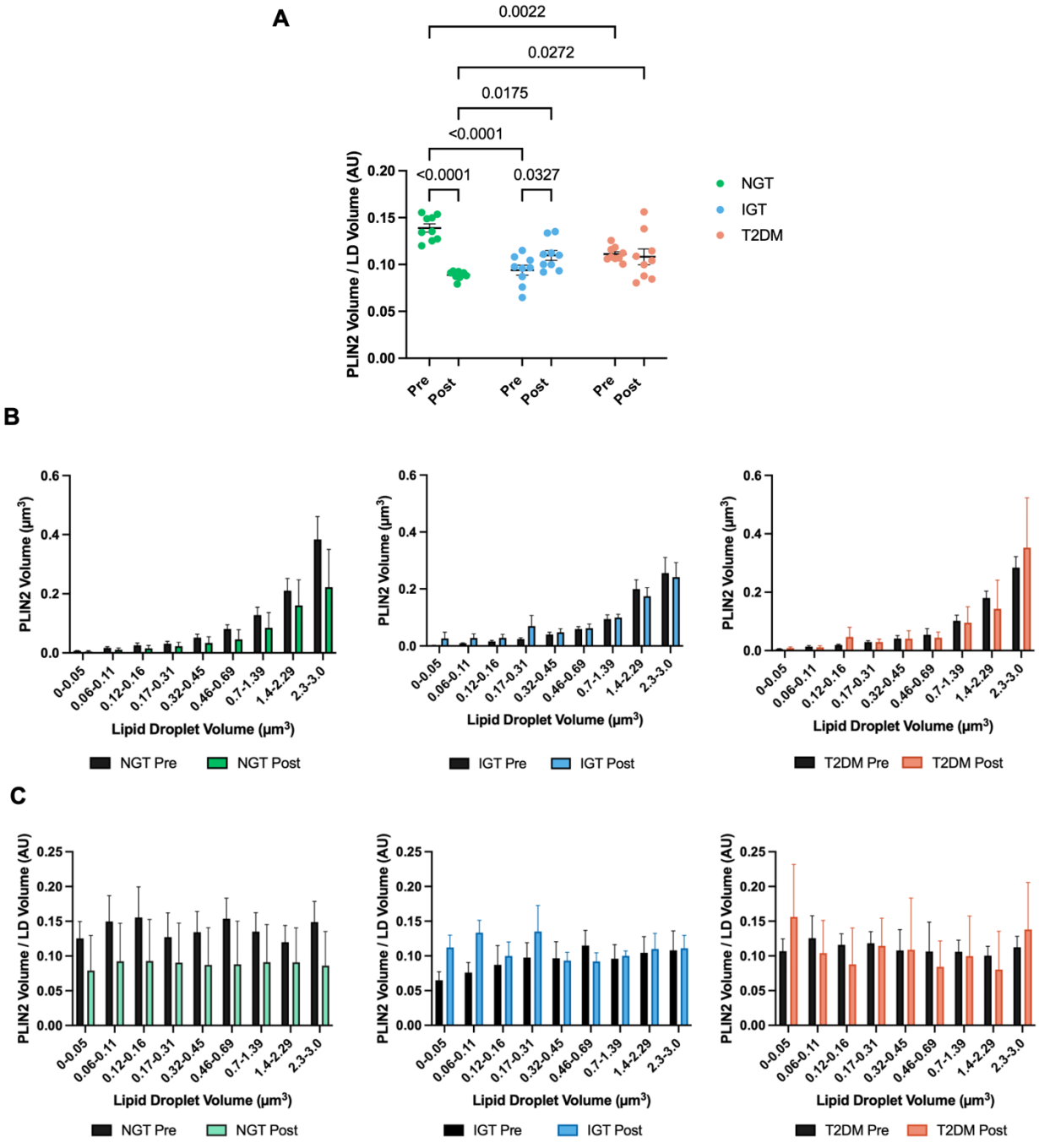


Figure 5. LD-associated PLIN2 by LD Size Group in Response to Training Across Metabolic Groups. (A) LD-associated PLIN2 by LD volume across groups, (B) absolute LD - associated PLIN2 expression by size bin, and (C) LD-associated PLIN2 by LD volume across LD size groups. Data are mean ± SEM.

Perilipin 5 and Lipid Droplet Morphology

LD-associated PLIN5 by LD size groups was measured to assess potential variations in expression and how that could relate to LD size. There was a glucose tolerance dependent effect of training on LD-associated PLIN5 by LD volume. Before training, the NGT group expressed 50% more LD-associated PLIN5 by LD volume than the IGT group ($p = 0.0078$, Figure 6A) but had similar LD-associated PLIN5 to the T2DM group ($p = 0.87$). Like the normal glucose tolerance group, the T2DM group expressed 79% more LD-associated PLIN5 by LD volume than the IGT group at baseline ($p = 0.022$). This finding may signify a transitional period where prediabetic muscle may lose PLIN5 mediated lipid utilization, where now T2DM muscle compensates by expressing greater PLIN5/LD volume in attempt to utilize lipids.

Following training, the NGT group had a 31% decrease in LD-associated PLIN5 by LD volume expression ($p = 0.038$, Figure 6A). Both groups with impaired glucose tolerance had an increase in LD-associated PLIN5 expression, as the IGT group had a 67% increase ($p = 0.02$) and the T2DM group increased PLIN5/LD volume by 54% ($p = 0.002$) in response to training. This pattern demonstrates that upregulation of LD-associated PLIN5 by LD volume is the most prominent in insulin resistant muscle. After training, the T2DM group expressed 100% greater LD-associated PLIN5 by LD volume than the NGT group ($p = 0.0002$) and 79% greater expression than the IGT group ($p = 0.02$). Additionally, this response to training is highlighted by the NGT and IGT groups no longer presenting with different LD-associated PLIN5 by LD volume ($p = 0.42$).

Across glucose tolerance groups, LD-associated PLIN5 increased with droplet size and there was mostly no effect of training on absolute expression across LD size groups. However, there was a significant decline in NGT absolute PLIN5 expression on large lipid droplets

between the size of 2.3-3.0μm^3 ($p = 0.004$, Figure 6B). This suggests glucose tolerant individuals who six months into an aerobic training program have a preferential reduction in PLIN5 expression on large LDs. Although not statistically significant, the variation in LD-associated PLIN5 by LD volume in the T2DM group compared to NGT and IGT was highly variable by subject (Figure 6C).

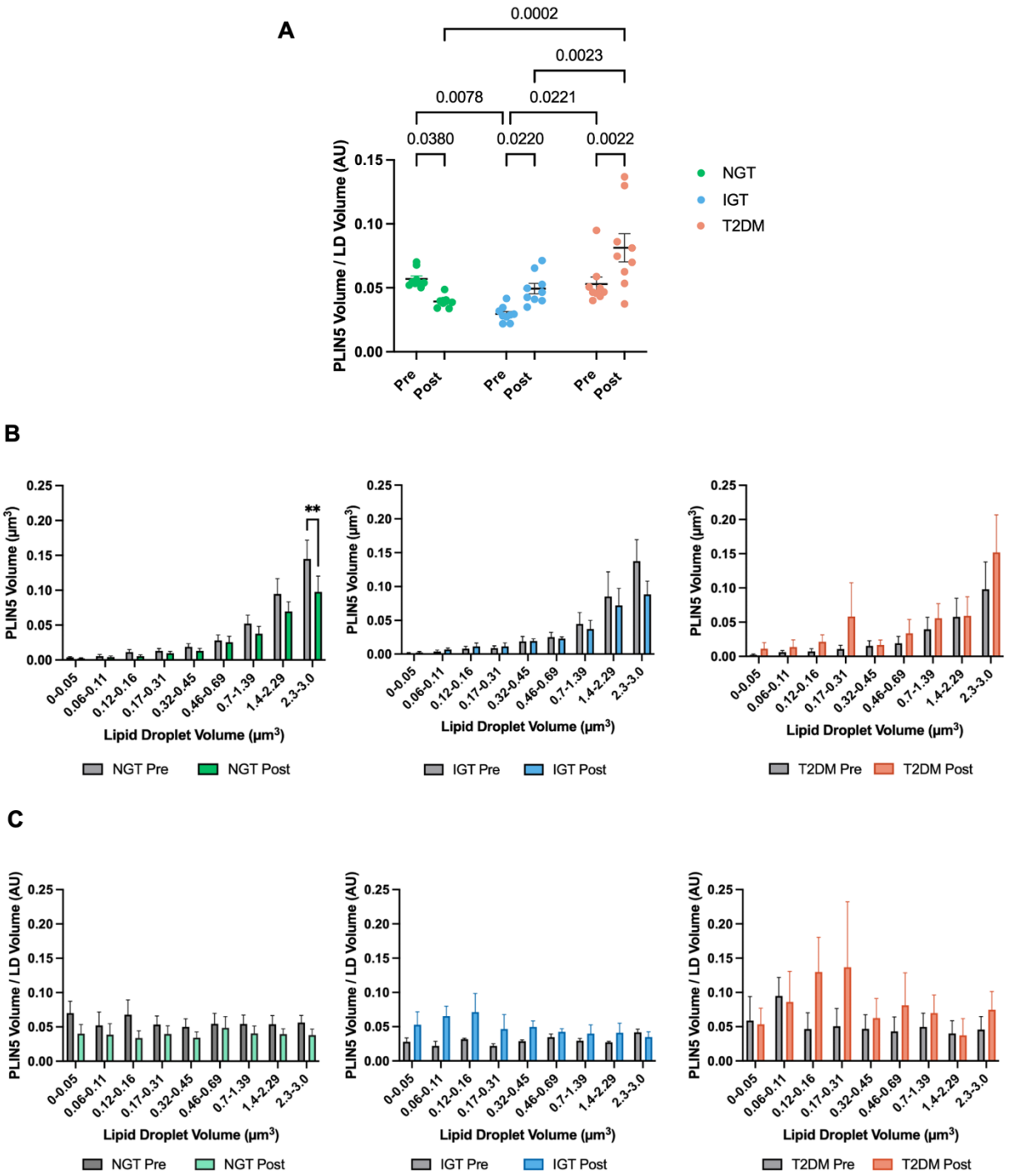


Figure 6. LD-associated PLIN5 by LD Size Group in Response to Training Across Metabolic Groups. (A) PLIN5 Volume normalized to total LD volume across groups, (B) absolute PLIN5 expression by size bin, and (C) PLIN5 volume normalized to LD volume across size bins. Data are mean ± SEM.

Chapter 6: Discussion

This study assessed the effects of insulin sensitivity status and six months of aerobic training on lipid droplet morphology and perilipin protein association in previously sedentary older adults. The principal findings of this study include: (1) total PLIN2 and PLIN5 protein expression was greater following training regardless of insulin sensitivity group; (2) LD-associated PLIN5 by LD volume increased to a greater extent in the T2DM group than in the NGT group after training, with a significant difference between NGT and T2DM following the intervention; (3) six months of aerobic training reduced LD-associated PLIN2 by LD volume in NGT while increasing in IGT, with no change in the T2DM group; and (4) LD-associated PLIN5 by LD volume decreased in NGT following training but increased in both IGT and T2DM groups, resulting in greater PLIN5 expression in T2DM compared to both NGT and IGT after training. Collectively, these findings indicate a glucose tolerance-dependent adaptation in PLIN and LD interaction in response to aerobic exercise training, where T2DM individuals display PLIN remodeling patterns towards improved lipid storage and utilization.

Skeletal Muscle PLIN Protein Expression

The observed training-induced increases in total skeletal muscle PLIN2 and PLIN5 protein expression are consistent with prior work in T2DM populations. A threefold increase in PLIN2 expression in T2DM type 1 muscle fibers along with a two-fold increase in lipid content has been previously reported following six months of aerobic exercise training in a T2DM population alone (Shaw et al., 2012). The current findings expand this work by demonstrating greater PLIN2 expression can be present across a spectrum of glucose tolerance groups. The absence of a group × training interaction on PLIN2 protein expression suggests that total PLIN2

upregulation with exercise may be a general skeletal muscle adaptation to training, rather than a mechanistic response only seen in insulin resistance. This expands upon the work reported by (Shaw et al., 2012) as the increase in PLIN2 reported in one T2DM group is seen across all groups in the current study.

A lack of group difference in PLIN2 protein expression at baseline, despite previously established associations in T2DM may reflect a limitation of homogenate-based western blotting to identify site-dependent protein interactions (Gemmink et al., 2021; Shaw et al., 2012). While PLIN2 is exclusively expressed on LDs, the total PLIN expression approach utilizing homogenates cannot differentiate between protein bound to lipid droplets in within a muscle fiber, or proteins bound to LDs within the sarcoplasmic reticulum (Sztalryd & Brasaemle, 2017). This highlights the distinction between homogenate and histological based analyses used in Aim 2 of this thesis.

The current 1.35-fold increase in total PLIN5 protein abundance combined with the greater training effect in T2DM compared to NGT individuals has not been previously reported across glucose tolerance groups in a longitudinal study of this kind. Greater PLIN5 protein content has been reported in a cross-sectional study with endurance-trained athletes, but this was in comparison to a T2DM population and was correlated with greater oxidative capacity in the endurance athletes (Laurens et al., 2016). However, this current study is the first to our knowledge, to demonstrate that PLIN5 upregulation is more pronounced in T2DM in response to training. This finding is consistent with the notion that PLIN5 upregulation may serve a protective role to preserve glucose tolerance in skeletal muscle. Additionally, this indicates that whole body oxidative capacity is not strongly related to PLIN5 expression, as there were no

differences in $\dot{V}O_{2max}$ between groups, but there was a greater expression of PLIN5 in whole T2DM skeletal muscle homogenates and type 1 LD-associated PLIN5 by LD volume.

Lipid Droplet Morphology and PLIN Protein Expression

At baseline, NGT participants had greater LD-associated PLIN2 by LD volume than both IGT and T2DM individuals, with no difference between IGT and T2DM groups. Since smaller LDs have a greater surface area relative to volume, LD-associated PLIN2 by LD volume has a greater potential for surface interactions as the same volume of lipid is distributed over a greater number of smaller LDs. This observation aligns with the work of (Gemmink et al., 2021), who reported that PLIN2 associated LDs were correlated with greater LD size in insulin-resistant muscle. However, this study compared lipid droplet volume and PLIN optical density in athletes and T2DM due to similarities in skeletal muscle lipid content. Based on the methods reported by (Gemmink et al., 2021), the current study design follows similar analysis and imaging protocols and with the additional third dimension instead of analyzing LDs from a two dimensional plane. In the current study design, lipid morphology and PLIN expression was assessed across groups with similar training history and body composition measurements.

The alignment of LD-associated PLIN2 by LD volume at baseline between IGT and T2DM in comparison to NGT suggests that a transitional phase from NGT to pre-diabetic glucose intolerance may be associated with greater expression of PLIN2 across droplets. Previous work has largely assessed PLIN2 expression in highly trained aerobic athletes and T2DM due to similarities in skeletal muscle lipid volume, but there is a vast difference in skeletal muscle oxidative capacity between groups (Gemmink et al., 2021). It has been previously demonstrated that skeletal muscle with PLIN2 knockdown lack the capacity to accumulate lipids and leads to greater total fatty acid oxidation (Bosma et al., 2012). This

mechanism may be seen at baseline in the current study, as the sedentary NGT individuals expressed greater LD-associated PLIN2 by LD volume likely due to the retained ability to regulate glucose and untrained individuals have a greater contribution of carbohydrates as a fuel source in a rested state (Mul et al., 2015). However, in the IGT and T2DM populations at baseline there is lower expression of LD-associated PLIN2 by LD volume, which may lead to the accumulation of lipid intermediates like DAGs and ceramides in attempt to meet energy demands with impaired skeletal muscle glucose uptake. This is a novel contribution of the current study, as previously reported work has not included an intermediate pre-diabetic cohort or assessed this outcome in groups across the glucose tolerance spectrum with similar training history. Clinically, this contribution represents a critical window to introduce training interventions for prediabetics before LD PLIN association is comparable to T2DM skeletal muscle.

In the present study, six months of aerobic training in previously sedentary individuals produced different effects on LD-associated PLIN2 by LD volume in a glucose tolerance dependent manner. The decline in LD-associated PLIN2 by LD volume in the NGT group is likely due to the training induced adaptations seen in $\dot{V}O_{2max}$ and support the belief that elevated LD-associated PLIN2 by LD volume was serving as a protective mechanism to meet energy demands while limiting the potential for partial lipid oxidation. Following training, and an increase in NGT $\dot{V}O_{2max}$ greater LD-associated PLIN2 by LD volume was no longer required as these lipids were now being effectively utilized at rest. From the current homogenate data, the increase in total PLIN2 expression was not related to the increase in LD-associated PLIN2 by LD volume seen in type 1 muscle fibers. This is consistent with known athlete skeletal muscle physiology where greater skeletal muscle lipid content was associated with lower PLIN2

associated with LDs (Daemen et al., 2018). Interestingly, the opposite response was observed as LD-associated PLIN2 by LD volume increased following training in IGT individuals. However, when comparing expression across groups, PLIN2 expression was significantly lower in IGT participants at baseline and the IGT group did not improve $\dot{V}O_{2max}$ during the training intervention.

The T2DM group had no significant change in LD-associated PLIN2 by LD volume, which may be due to the low volume already reported at the start of the training intervention. Additionally, many of the T2DM participants were taking metformin, which increases AMPK activity and the potential for AMPK mediated glucose uptake in skeletal muscle, which is preserved in T2DM individuals (Lund et al., 1995; Musi et al., 2002). While total PLIN2 protein expression increased with training, this was not reflected in LD-associated PLIN2 by LD volume in type 1 muscle fibers across groups.

The response to training in LD-associated PLIN5 by LD volume was also dependent on glucose tolerance status. NGT individuals had a LD-associated PLIN5 by LD volume decrease following training, which may be due to the reduction in LD-associated PLIN2 and competitive inhibition of lipid tethering. In IGT and T2DM individuals, LD-associated PLIN5 by LD volume increased from baseline. Work by (Gemmink et al., 2018) proposed greater PLIN5 coating per droplet may aid in LD tethering to mitochondria to enhance the transfer of free fatty acids. The current study is the first to demonstrate this adaptive mechanism in glucose intolerant skeletal muscle in response to training. This is also consistent with the more widely known relationship between whole body maximal oxygen uptake and muscle fiber oxidative capacity (Van Der Zwaard et al., 2016). After training, the T2DM group expressed greater LD-associated PLIN5 by LD volume than both NGT and IGT groups. Although both insulin resistant groups had greater

LD-associated PLIN5 by LD volume, the effect of training was greater in the T2DM group than individuals with IGT.

PLIN association scaled proportionally across most groups by LD volume. The only exception was lower PLIN5 volume in the NGT group after training, and only on LDs in the 2.3-3.0μm^3 size range. This aligns with preferential utilization of large LDs that can be seen after a single aerobic exercise bout (Bajpeyi et al., 2023). In a longitudinal training study, this pattern has not been previously described using high resolution microscopy at the droplet level. As this only happened in the NGT group, this could indicate that the preferential expression of PLIN5 on large droplets may be dependent on a combination of adequate oxidative capacity and potential for skeletal muscle glucose uptake.

Limitations

Several limitations should be considered when interpreting the findings of the current study. First, sample sizes across groups are relatively small, and fewer samples were used in histological analyses. The power analysis for this study was conducted using previously reported lipid droplet diameters, but a conversion was still used as no prior data existed for the primary outcomes. However, results from the current study at the droplet level still indicate these relationships are highly correlated. Future studies with larger cohorts would be needed to confirm some of the current findings presented in this study, including the lack of significance in T2DM LD-associated PLIN2 by LD volume in response to training.

Second, differentiation between subsarcolemmal and intermyofibrillar lipid droplets without a laminin or dystrophin was not assessed in the current study. The current analysis still holds physiological relevance as it identifies regions where skeletal muscle lipid oxidation is the most likely to contribute to energy requirements relating to contraction. However, this does not

account for differences in location dependent mitochondrial actions or mitochondrial networks connecting subsarcolemmal and IMF regions (Bleck et al., 2018). Relative $\dot{V}O_{2max}$ was used as an alternate measure for oxidative capacity, but this is a measure of oxygen uptake instead of skeletal muscle specific oxidative capacity (Van Der Zwaard et al., 2016). The interpretation that LD-associated PLIN5 by LD volume is due to greater mitochondrial content remains mechanistically plausible but needs to be confirmed.

Finally, longitudinal human research studies introduce variability that cannot be entirely controlled. Dietary intake was monitored and participants were instructed to maintain a certain percentage of their body weight, but participants did lose weight while going through the training intervention. Individuals in the T2DM group were also on medication throughout the duration of the study. Due to sample size, separate analyses could not be conducted to rule out the potential influence of medications like statins, metformin and other glucose-lowering medications like insulin on lipid droplet morphology. However, the prediabetic cohort, which did not include any participants on medication, demonstrated response patterns that translate to the T2DM group.

Future Directions

Future studies may evaluate the shape and volume of lipid droplets by droplet size. It has been previously demonstrated that the shape of lipid droplets could indicate the metabolic activity status of that droplets (Nielsen et al., 2017). In this case, elongated droplets have been more frequently identified in oxidative type 1 skeletal muscle, while round droplets were more common in more glycolytic fiber types. Additionally, the quantification of mitochondrial content through transmission electron microscopy paired with PLIN localization would provide information on the relationship between PLIN expression and mitochondrial interactions. While differences in PLIN expression are reported here, these findings are not directly related to

mitochondrial content. By assessing these interactions in conjunction with mitochondrial networks supplying contractile elements, there would be a more complete understanding of the contributions of each individual LD.

Another valuable addition to this study would be the inclusion of a resistance training or cross-training programs to compare training modalities on lipid droplet morphology and perilipin association. Evidence suggests lipid droplet morphology varies between aerobic and resistance training, but the effects of resistance training has not been fully characterized with a spectrum of glucose tolerance groups. It has been reported that aerobic training demonstrates greater improvements in total body fat reduction and lipid metabolism, but this may not apply across demographics (Musi et al., 2002; Yang et al., 2014; Zhao et al., 2025). Since resistance training causes a greater degree of skeletal muscle damage, this could clarify if total PLIN2 expression is increasing to aid in skeletal muscle repair (Lund et al., 1995). Additionally, resistance exercise initiates a greater inflammatory response, blunting the rate of glucose disposal (Kirwan et al., 1992), which could increase the threshold required for skeletal muscle glucose uptake, thus requiring skeletal muscle lipid utilization.

Conclusions

This thesis provides novel insight on the relationship between glucose tolerance and training status on LD morphology and PLIN protein association in previously-sedentary older adults. At baseline, NGT individuals express greater LD-associated PLIN2 by LD volume than both IGT and T2DM groups, which suggests that the preservation of glucose tolerance may be associated with the preservation of LDs in populations with low physical activity status. Following six months of training, LD-associated PLIN by LD volume varied by insulin sensitivity status. The greatest magnitude of LD-associated PLIN5 increase in response to

training was in the T2DM group followed by the IGT group, while the NGT group saw a decrease in expression. Together, these findings suggest that perilipin 2 and perilipin 5 play distinct roles in skeletal muscle metabolism. Perilipin 2 appears to help maintain glucose regulation in sedentary muscle, while perilipin 5 promotes lipid oxidation in muscle as an adaptation through aerobic exercise training.

Kinesiology Research and the School of Public Health

The field of kinesiology explores the impact of human movement and health measured through biological, behavioral and social perspectives. The foundation of kinesiology is built on research addressing changes to body functions in response to exercise and movement. Along with this, kinesiology focuses on building wholistic approaches to promote health, increase health-span and enhance quality of life. The study of skeletal muscle metabolism and insulin sensitivity is an example of how molecular differences can be related to greater public health outcomes.

This thesis addresses the importance of physical activity on whole body glucose regulation and the impact of exercise training on lipid utilization in muscle. Skeletal muscle is key site for glucose clearance and plays a critical role in insulin sensitivity status. While living a sedentary lifestyle, obesity and impaired mitochondrial function contribute to insulin resistance and T2DM. Both obesity and T2DM are major public health concerns that continue to rise around the world. These conditions highlight the need for research on this topic to better promote physical activity. This is done by examining cellular-based differences that can be identified in response to aerobic exercise training. Promotion of physical activity can range from individualized clinical care plans to community-based interventions to prevent physical inactivity.

The current research study primarily falls under the discipline of exercise physiology, but addresses aspects of biomechanics, psychology and social determinants of public health. By identify molecular alterations with chronic aerobic exercise, public health goals can be established to address cross discipline goals for greater public health outcomes. Public health outcomes should focus on decreasing physical inactivity by encouraging movement through

various avenues when access to exercising facilities is limited. The subfield of cardiometabolic mechanisms focuses on how the body utilizes multiple fuel sources to meet energy demands, especially in response to exercise. Within current literature, lipid droplet morphology and perilipin proteins are used to understand the importance of exercise in the development of T2DM. Differences in any of these components can have direct effects on T2DM prevention and how treatment plans are presented. Exercise training has consistently shown to improve insulin sensitivity, yet individuals with insulin resistance do not understand the importance of movement in disease prevention.

The University of Maryland School of Public Health (SPH) aims to advance current research and translate these findings to promote better health and overall well-being. This is accomplished through improvements in health equity, education and community engagement. The overarching goal of this current project is to address the progression of chronic disease and identify sustainable lifestyle modifications.

Linking molecular signaling pathways in skeletal muscle metabolism with the relevance to public health aligns with the commitment of SPH to translate knowledge for interdisciplinary research. The research outlined in this thesis directly aligns with SPH initiatives to promote physical activity, exercise education, and creation of community-based interventions. Through a better understanding of lipid droplet morphology, community-based workout programs can be built that target cellular mechanisms while also creating an environment where people want to continue physical activity. To remain engaged in workout programs, people seek validation that the effort spent on physical activity is improving their overall wellbeing. Additionally, the creation of workout groups creates a sense of belonging, which is crucial in the psychological adherence to exercise training programs.

The interconnection between skeletal muscle function, insulin sensitivity and physical activity is a clear example of how kinesiology is connected from molecular mechanisms through behavioral sciences. At the molecular level, the dynamics of lipid droplet proteins and lipid droplet morphology determine the effectiveness of skeletal muscle fibers to uptake glucose and store energy. At the behavioral level, these mechanisms may also be used to assess why some individuals live a lifestyle of activity avoidance. Activity avoidance can be due to factors like aging, lack of self-belief and lack of belonging within a community. Practitioners and policymakers can use these mechanisms to manage metabolic disorders like T2DM and develop wholistic physical activity guidelines that are region specific. Examples of this could be improving bike routes in highly populated regions, providing swimming lessons for communities based near water or improving access to winter sports equipment for participation in activities during cold months. These three workout modalities can be adapted to various physical limitations and accomplish the goal of promoting aerobic physical activity.

This thesis demonstrates an understanding of kinesiology as an interdisciplinary science that acknowledges how differences between populations, regions and beliefs contribute to the broader context of public health. By assessing how physical activity regulates glucose metabolism at the molecular level, this work contributes to a greater understanding of how exercise can be used as a therapeutic strategy in preventative medicine. In alignment with the University of Maryland School of Public Health, this research highlights the importance of physical activity as a modifiable chronic disease risk factor. Physical activity can enhance metabolic health, reduce disease risk and improve health span across populations at various stages in life. The aim of this thesis is to identify differences in perilipin protein association and

lipid droplet morphology across a range of individuals with varying insulin sensitivity statuses, then to determine variations in training response.

Bibliography

AbouAssi, H., Slentz, C. A., Mikus, C. R., Tanner, C. J., Bateman, L. A., Willis, L. H., Shields, A. T., Piner, L. W., Penry, L. E., Kraus, E. A., Huffman, K. M., Bales, C. W., Houmard, J. A., & Kraus, W. E. (2015). The effects of aerobic, resistance, and combination training on insulin sensitivity and secretion in overweight adults from STRRIDE AT/RT: A randomized trial. *Journal of Applied Physiology*, *118*(12), 1474–1482. https://doi.org/10.1152/japplphysiol.00509.2014

Aguirre, V., Uchida, T., Yenush, L., Davis, R., & White, M. F. (2000). The c-Jun NH2-terminal Kinase Promotes Insulin Resistance during Association with Insulin Receptor Substrate-1 and Phosphorylation of Ser307. *Journal of Biological Chemistry*, *275*(12), 9047–9054. https://doi.org/10.1074/jbc.275.12.9047

Aguirre, V., Werner, E. D., Giraud, J., Lee, Y. H., Shoelson, S. E., & White, M. F. (2002). Phosphorylation of Ser307 in Insulin Receptor Substrate-1 Blocks Interactions with the Insulin Receptor and Inhibits Insulin Action. *Journal of Biological Chemistry*, *277*(2), 1531–1537. https://doi.org/10.1074/jbc.M101521200

American Diabetes Association. (2012). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, *35*(Supplement_1), S64–S71. https://doi.org/10.2337/dc12-s064

Arner, P., & Rydén, M. (2015). Fatty Acids, Obesity and Insulin Resistance. *Obesity Facts*, *8*(2), 147–155. https://doi.org/10.1159/000381224

Bajpeyi, S., Apaflo, J. N., Rosas, V., Sepulveda-Rivera, K., Varela-Ramirez, A., Covington, J. D., Galgani, J. E., & Ravussin, E. (2023). Effect of an acute long-duration exercise bout on skeletal muscle lipid droplet morphology, GLUT 4 protein, and perilipin protein expression. *European Journal of Applied Physiology*, *123*(12), 2771–2778. https://doi.org/10.1007/s00421-023-05266-5

Baron, A. D., Brechtel, G., Wallace, P., & Edelman, S. V. (1988). Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *American Journal of Physiology-Endocrinology and Metabolism*, *255*(6), E769–E774. https://doi.org/10.1152/ajpendo.1988.255.6.E769

Bellou, V., Belbasis, L., Tzoulaki, I., & Evangelou, E. (2018). Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. *PLOS ONE*, *13*(3), e0194127. https://doi.org/10.1371/journal.pone.0194127

Berg, S., Kutra, D., Kroeger, T., Straehle, C. N., Kausler, B. X., Haubold, C., Schiegg, M., Ales, J., Beier, T., Rudy, M., Eren, K., Cervantes, J. I., Xu, B., Beuttenmueller, F., Wolny, A., Zhang, C., Koethe, U., Hamprecht, F. A., & Kreshuk, A. (2019). ilastik: Interactive machine learning for (bio)image analysis. *Nature Methods*, *16*(12), 1226–1232. https://doi.org/10.1038/s41592-019-0582-9

Bergman, B. C., & Goodpaster, B. H. (2020). Exercise and Muscle Lipid Content, Composition, and Localization: Influence on Muscle Insulin Sensitivity. *Diabetes*, *69*(5), 848–858. https://doi.org/10.2337/dbi18-0042

Bleck, C. K. E., Kim, Y., Willingham, T. B., & Glancy, B. (2018). Subcellular connectomic analyses of energy networks in striated muscle. *Nature Communications*, *9*(1), 5111. https://doi.org/10.1038/s41467-018-07676-y

Bosma, M., Hesselink, M. K. C., Sparks, L. M., Timmers, S., Ferraz, M. J., Mattijssen, F., Van Beurden, D., Schaart, G., De Baets, M. H., Verheyen, F. K., Kersten, S., & Schrauwen, P. (2012). Perilipin 2 Improves Insulin Sensitivity in Skeletal Muscle Despite Elevated Intramuscular Lipid Levels. *Diabetes*, *61*(11), 2679–2690. https://doi.org/10.2337/db11-1402

Cacicedo, J. M., Gauthier, M.-S., Lebrasseur, N. K., Jasuja, R., Ruderman, N. B., & Ido, Y. (2011). Acute exercise activates AMPK and eNOS in the mouse aorta. *American Journal of Physiology-Heart and Circulatory Physiology*, *301*(4), H1255–H1265. https://doi.org/10.1152/ajpheart.01279.2010

CDC. (2024, September 25). *Type 2 Diabetes*. Diabetes. https://www.cdc.gov/diabetes/about/about-type-2-diabetes.html

Coen, P. M., Hames, K. C., Leachman, E. M., DeLany, J. P., Ritov, V. B., Menshikova, E. V., Dubé, J. J., Stefanovic-Racic, M., Toledo, F. G. S., & Goodpaster, B. H. (2013). Reduced skeletal muscle oxidative capacity and elevated ceramide but not diacylglycerol content in severe obesity: Muscle Oxidative Capacity in Obesity. *Obesity*, *21*(11), 2362–2371. https://doi.org/10.1002/oby.20381

Conte, M., Vasuri, F., Trisolino, G., Bellavista, E., Santoro, A., Degiovanni, A., Martucci, E., D'Errico-Grigioni, A., Caporossi, D., Capri, M., Maier, A. B., Seynnes, O., Barberi, L., Musarò, A., Narici, M. V., Franceschi, C., & Salvioli, S. (2013). Increased Plin2 Expression in Human Skeletal Muscle Is Associated with Sarcopenia and Muscle Weakness. *PLoS ONE*, *8*(8), e73709. https://doi.org/10.1371/journal.pone.0073709

Daemen, S., Gemmink, A., Brouwers, B., Meex, R. C. R., Huntjens, P. R., Schaart, G., Moonen-Kornips, E., Jörgensen, J., Hoeks, J., Schrauwen, P., & Hesselink, M. K. C. (2018). Distinct lipid droplet characteristics and distribution unmask the apparent contradiction of the athlete's paradox. *Molecular Metabolism*, *17*, 71–81. https://doi.org/10.1016/j.molmet.2018.08.004

DeFronzo, R. A., Jacot, E., Jequier, E., Maeder, E., Wahren, J., & Felber, J. P. (1981). The Effect of Insulin on the Disposal of Intravenous Glucose: Results from Indirect Calorimetry and

Hepatic and Femoral Venous Catheterization. *Diabetes*, *30*(12), 1000–1007. https://doi.org/10.2337/diab.30.12.1000

DeFronzo, R. A., & Tripathy, D. (2009). Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes. *Diabetes Care*, *32*(suppl_2), S157–S163. https://doi.org/10.2337/dc09-S302

Domander, R., Felder, A., & Doube, M. (2021). *BoneJ2—Refactoring established research software*. https://doi.org/10.12688

Evans, P. L., McMillin, S. L., Weyrauch, L. A., & Witczak, C. A. (2019). Regulation of Skeletal Muscle Glucose Transport and Glucose Metabolism by Exercise Training. *Nutrients*, *11*(10), 2432. https://doi.org/10.3390/nu11102432

Fachada, V., Rahkila, P., Fachada, N., Turpeinen, T., Kujala, U. M., & Kainulainen, H. (2022). Enlarged PLIN5-uncoated lipid droplets in inner regions of skeletal muscle type II fibers associate with type 2 diabetes. *Acta Histochemica*, *124*(3), 151869. https://doi.org/10.1016/j.acthis.2022.151869

Gemmink, A., Daemen, S., Brouwers, B., Hoeks, J., Schaart, G., Knoops, K., Schrauwen, P., & Hesselink, M. K. C. (2021). Decoration of myocellular lipid droplets with perilipins as a marker for in vivo lipid droplet dynamics: A super-resolution microscopy study in trained athletes and insulin resistant individuals. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1866*(2), 158852. https://doi.org/10.1016/j.bbalip.2020.158852

Gemmink, A., Daemen, S., Kuijpers, H. J. H., Schaart, G., Duimel, H., López-Iglesias, C., Van Zandvoort, M. A. M. J., Knoops, K., & Hesselink, M. K. C. (2018). Super-resolution microscopy localizes perilipin 5 at lipid droplet-mitochondria interaction sites and at lipid

droplets juxtaposing to perilipin 2. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1863*(11), 1423–1432. https://doi.org/10.1016/j.bbalip.2018.08.016

Gemmink, A., Goodpaster, B. H., Schrauwen, P., & Hesselink, M. K. C. (2017). Intramyocellular lipid droplets and insulin sensitivity, the human perspective. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1862*(10), 1242–1249. https://doi.org/10.1016/j.bbalip.2017.07.010

Genders, A. J., Holloway, G. P., & Bishop, D. J. (2020). Are Alterations in Skeletal Muscle Mitochondria a Cause or Consequence of Insulin Resistance? *International Journal of Molecular Sciences*, *21*(18), 6948. https://doi.org/10.3390/ijms21186948

Goodpaster, B. H., He, J., Watkins, S., & Kelley, D. E. (2001). *Skeletal Muscle Lipid Content and Insulin Resistance: Evidence for a Paradox in Endurance-Trained Athletes*.

Harris, L.-A. L. S., Skinner, J. R., Shew, T. M., Pietka, T. A., Abumrad, N. A., & Wolins, N. E. (2015). Perilipin 5–Driven Lipid Droplet Accumulation in Skeletal Muscle Stimulates the Expression of Fibroblast Growth Factor 21. *Diabetes*, *64*(8), 2757–2768. https://doi.org/10.2337/db14-1035

Hilder, T. L., Tou, J. C. L., Grindeland, R. E., Wade, C. E., & Graves, L. M. (2003). Phosphorylation of insulin receptor substrate-1 serine 307 correlates with JNK activity in atrophic skeletal muscle. *FEBS Letters*, *553*(1–2), 63–67. https://doi.org/10.1016/S0014-5793(03)00972-4

Høeg, L., Roepstorff, C., Thiele, M., Richter, E. A., Wojtaszewski, J. F. P., & Kiens, B. (2009). Higher intramuscular triacylglycerol in women does not impair insulin sensitivity and

proximal insulin signaling. *Journal of Applied Physiology*, *107*(3), 824–831. https://doi.org/10.1152/japplphysiol.91382.2008

Hood, D. A. (2001). Invited Review: Contractile activity-induced mitochondrial biogenesis in skeletal muscle. *Journal of Applied Physiology*, *90*(3), 1137–1157. https://doi.org/10.1152/jappl.2001.90.3.1137

Jacob, S., Machann, J., Rett, K., Brechtel, K., Volk, A., Renn, W., Maerker, E., Matthaei, S., Schick, F., Claussen, C. D., & Häring, H. U. (1999). Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes*, *48*(5), 1113–1119. https://doi.org/10.2337/diabetes.48.5.1113

Jorge, M. L. M. P., De Oliveira, V. N., Resende, N. M., Paraiso, L. F., Calixto, A., Diniz, A. L. D., Resende, E. S., Ropelle, E. R., Carvalheira, J. B., Espindola, F. S., Jorge, P. T., & Geloneze, B. (2011). The effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with type 2 diabetes mellitus. *Metabolism*, *60*(9), 1244–1252. https://doi.org/10.1016/j.metabol.2011.01.006

Kanaley, J. A., Shadid, S., Sheehan, M. T., Guo, Z., & Jensen, M. D. (2009). Relationship between plasma free fatty acid, intramyocellular triglycerides and long-chain acylcarnitines in resting humans. *The Journal of Physiology*, *587*(24), 5939–5950. https://doi.org/10.1113/jphysiol.2009.180695

Kazantzis, M., & Stahl, A. (2012). Fatty acid transport proteins, implications in physiology and disease. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1821*(5), 852–857. https://doi.org/10.1016/j.bbalip.2011.09.010

Kirwan, J. P., Hickner, R. C., Yarasheski, K. E., Kohrt, W. M., Wiethop, B. V., & Holloszy, J. O. (1992). Eccentric exercise induces transient insulin resistance in healthy individuals. *Journal of Applied Physiology*, *72*(6), 2197–2202. https://doi.org/10.1152/jappl.1992.72.6.2197

Knudsen, J. R., Steenberg, D. E., Hingst, J. R., Hodgson, L. R., Henriquez-Olguin, C., Li, Z., Kiens, B., Richter, E. A., Wojtaszewski, J. F. P., Verkade, P., & Jensen, T. E. (2020). Prior exercise in humans redistributes intramuscular GLUT4 and enhances insulin-stimulated sarcolemmal and endosomal GLUT4 translocation. *Molecular Metabolism*, *39*, 100998. https://doi.org/10.1016/j.molmet.2020.100998

Laurens, C., Bourlier, V., Mairal, A., Louche, K., Badin, P.-M., Mouisel, E., Montagner, A., Marette, A., Tremblay, A., Weisnagel, J. S., Guillou, H., Langin, D., Joanisse, D. R., & Moro, C. (2016). Perilipin 5 fine-tunes lipid oxidation to metabolic demand and protects against lipotoxicity in skeletal muscle. *Scientific Reports*, *6*(1), 38310. https://doi.org/10.1038/srep38310

Lee, H., & Song, W. (2018). Exercise and Mitochondrial Remodeling in Skeletal Muscle in Type 2 Diabetes. *Journal of Obesity & Metabolic Syndrome*, *27*(3), 150–157. https://doi.org/10.7570/jomes.2018.27.3.150

Legland, D., Arganda-Carreras, I., & Andrey, P. (2016). MorphoLibJ: Integrated library and plugins for mathematical morphology with ImageJ. *Bioinformatics*, *32*(22), 3532–3534. https://doi.org/10.1093/bioinformatics/btw413

Lund, S., Holman, G. D., Schmitz, O., & Pedersen, O. (1995). Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism

distinct from that of insulin. *Proceedings of the National Academy of Sciences*, *92*(13), 5817–5821. https://doi.org/10.1073/pnas.92.13.5817

Ma, X., Nan, F., Liang, H., Shu, P., Fan, X., Song, X., Hou, Y., & Zhang, D. (2022). Excessive intake of sugar: An accomplice of inflammation. *Frontiers in Immunology*, *13*, 988481. https://doi.org/10.3389/fimmu.2022.988481

Martin, I. K., Katz, A., & Wahren, J. (1995). Splanchnic and muscle metabolism during exercise in NIDDM patients. *American Journal of Physiology-Endocrinology and Metabolism*, *269*(3), E583–E590. https://doi.org/10.1152/ajpendo.1995.269.3.E583

Mason, R. R., Meex, R. C. R., Russell, A. P., Canny, B. J., & Watt, M. J. (2014). Cellular Localization and Associations of the Major Lipolytic Proteins in Human Skeletal Muscle at Rest and during Exercise. *PLoS ONE*, *9*(7), e103062. https://doi.org/10.1371/journal.pone.0103062

Mason, R. R., Mokhtar, R., Matzaris, M., Selathurai, A., Kowalski, G. M., Mokbel, N., Meikle, P. J., Bruce, C. R., & Watt, M. J. (2014). PLIN5 deletion remodels intracellular lipid composition and causes insulin resistance in muscle. *Molecular Metabolism*, *3*(6), 652–663. https://doi.org/10.1016/j.molmet.2014.06.002

Merz, K. E., & Thurmond, D. C. (2020). Role of Skeletal Muscle in Insulin Resistance and Glucose Uptake. In R. Terjung (Ed.), *Comprehensive Physiology* (1st ed., pp. 785–809). Wiley. https://doi.org/10.1002/cphy.c190029

Mikines, K. J., Sonne, B., Farrell, P. A., Tronier, B., & Galbo, H. (1988). Effect of physical exercise on sensitivity and responsiveness to insulin in humans. *American Journal of Physiology-Endocrinology and Metabolism*, *254*(3), E248–E259. https://doi.org/10.1152/ajpendo.1988.254.3.E248

Mohktar, R. A. M., Montgomery, M. K., Murphy, R. M., & Watt, M. J. (2016). Perilipin 5 is dispensable for normal substrate metabolism and in the adaptation of skeletal muscle to exercise training. *American Journal of Physiology-Endocrinology and Metabolism*, *311*(1), E128–E137. https://doi.org/10.1152/ajpendo.00084.2016

Morales, P. E., Bucarey, J. L., & Espinosa, A. (2017). Muscle Lipid Metabolism: Role of Lipid Droplets and Perilipins. *Journal of Diabetes Research*, *2017*, 1–10. https://doi.org/10.1155/2017/1789395

Mul, J. D., Stanford, K. I., Hirshman, M. F., & Goodyear, L. J. (2015). Exercise and Regulation of Carbohydrate Metabolism. In *Progress in Molecular Biology and Translational Science* (Vol. 135, pp. 17–37). Elsevier. https://doi.org/10.1016/bs.pmbts.2015.07.020

Musi, N., Hirshman, M. F., Nygren, J., & Svanfeldt, M. (2002). *Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes*. *51*(7), 2074–2081.

Nassis, G. P., Papantakou, K., Skenderi, K., Triandafillopoulou, M., Kavouras, S. A., Yannakoulia, M., Chrousos, G. P., & Sidossis, L. S. (2005). Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism*, *54*(11), 1472–1479. https://doi.org/10.1016/j.metabol.2005.05.013

Nielsen, J., Christensen, A. E., Nellemann, B., & Christensen, B. (2017). Lipid droplet size and location in human skeletal muscle fibers are associated with insulin sensitivity. *American Journal of Physiology-Endocrinology and Metabolism*, *313*(6), E721–E730. https://doi.org/10.1152/ajpendo.00062.2017

Nielsen, J., Mogensen, M., Vind, B. F., Sahlin, K., Højlund, K., Schrøder, H. D., & Ørtenblad, N. (2009). Increased subsarcolemmal lipids in type 2 diabetes: Effect of training on localization of lipids, mitochondria, and glycogen in sedentary human skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism*, *298*(3), E706–E713. https://doi.org/10.1152/ajpendo.00692.2009

Parry, H. A., & Glancy, B. (2021). Energy transfer between the mitochondrial network and lipid droplets in insulin resistant skeletal muscle. *Current Opinion in Physiology*, *24*, 100487. https://doi.org/10.1016/j.cophys.2022.100487

Phielix, E., Meex, R., Moonen-Kornips, E., Hesselink, M. K. C., & Schrauwen, P. (2010). Exercise training increases mitochondrial content and ex vivo mitochondrial function similarly in patients with type 2 diabetes and in control individuals. *Diabetologia*, *53*(8), 1714–1721. https://doi.org/10.1007/s00125-010-1764-2

Ploug, T., Galbo, H., & Richter, E. A. (1984). Increased muscle glucose uptake during contractions: No need for insulin. *American Journal of Physiology-Endocrinology and Metabolism*, *247*(6), E726–E731. https://doi.org/10.1152/ajpendo.1984.247.6.E726

Prior, S. J., Blumenthal, J. B., Katzel, L. I., Goldberg, A. P., & Ryan, A. S. (2014). Increased Skeletal Muscle Capillarization After Aerobic Exercise Training and Weight Loss Improves Insulin Sensitivity in Adults With IGT. *Diabetes Care*, *37*(5), 1469–1475. https://doi.org/10.2337/dc13-2358

Prior, S. J., Goldberg, A. P., Ortmeyer, H. K., Chin, E. R., Chen, D., Blumenthal, J. B., & Ryan, A. S. (2015). Increased Skeletal Muscle Capillarization Independently Enhances Insulin Sensitivity in Older Adults After Exercise Training and Detraining. *Diabetes*, *64*(10), 3386–3395. https://doi.org/10.2337/db14-1771

Richter, E. A., Bilan, P. J., & Klip, A. (2025). A comprehensive view of muscle glucose uptake: Regulation by insulin, contractile activity, and exercise. *Physiological Reviews*, *105*(3), 1867–1945. https://doi.org/10.1152/physrev.00033.2024

Seibert, J. T., Najt, C. P., Heden, T. D., Mashek, D. G., & Chow, L. S. (2020). Muscle Lipid Droplets: Cellular Signaling to Exercise Physiology and Beyond. *Trends in Endocrinology & Metabolism*, *31*(12), 928–938. https://doi.org/10.1016/j.tem.2020.08.002

Shaw, C. S., Shepherd, S. O., Wagenmakers, A. J. M., Hansen, D., Dendale, P., & Van Loon, L. J. C. (2012). Prolonged exercise training increases intramuscular lipid content and perilipin 2 expression in type I muscle fibers of patients with type 2 diabetes. *American Journal of Physiology-Endocrinology and Metabolism*, *303*(9), E1158–E1165. https://doi.org/10.1152/ajpendo.00272.2012

Shojaee-Moradie, F., Baynes, K. C. R., Pentecost, C., Bell, J. D., Thomas, E. L., Jackson, N. C., Stolinski, M., Whyte, M., Lovell, D., Bowes, S. B., Gibney, J., Jones, R. H., & Umpleby, A. M. (2007). Exercise training reduces fatty acid availability and improves the insulin sensitivity of glucose metabolism. *Diabetologia*, *50*(2), 404–413. https://doi.org/10.1007/s00125-006-0498-7

Short, K. R., Vittone, J. L., Bigelow, M. L., Proctor, D. N., Rizza, R. A., Coenen-Schimke, J. M., & Nair, K. S. (2003). Impact of Aerobic Exercise Training on Age-Related Changes in Insulin Sensitivity and Muscle Oxidative Capacity. *Diabetes*, *52*(8), 1888–1896. https://doi.org/10.2337/diabetes.52.8.1888

Stanford, K. I., & Goodyear, L. J. (2014). Exercise and type 2 diabetes: Molecular mechanisms regulating glucose uptake in skeletal muscle. *Advances in Physiology Education*, *38*(4), 308–314. https://doi.org/10.1152/advan.00080.2014

Sztalryd, C., & Brasaemle, D. L. (2017). The perilipin family of lipid droplet proteins: Gatekeepers of intracellular lipolysis. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1862*(10), 1221–1232. https://doi.org/10.1016/j.bbalip.2017.07.009

Tøien, T., Nielsen, J. L., Berg, O. K., Brobakken, M. F., Nyberg, S. K., Espedal, L., Malmo, T., Frandsen, U., Aagaard, P., & Wang, E. (2023). The impact of life-long strength versus endurance training on muscle fiber morphology and phenotype composition in older men. *Journal of Applied Physiology*, *135*(6), 1360–1371. https://doi.org/10.1152/japplphysiol.00208.2023

Van Der Zwaard, S., De Ruiter, C. J., Noordhof, D. A., Sterrenburg, R., Bloemers, F. W., De Koning, J. J., Jaspers, R. T., & Van Der Laarse, W. J. (2016). Maximal oxygen uptake is proportional to muscle fiber oxidative capacity, from chronic heart failure patients to professional cyclists. *Journal of Applied Physiology*, *121*(3), 636–645. https://doi.org/10.1152/japplphysiol.00355.2016

Walther, T. C., & Farese, R. V. (2012). Lipid Droplets and Cellular Lipid Metabolism. *Annual Review of Biochemistry*, *81*(1), 687–714. https://doi.org/10.1146/annurev-biochem-061009-102430

Whytock, K. L., & Goodpaster, B. H. (2025). Unraveling Skeletal Muscle Insulin Resistance: Molecular Mechanisms and the Restorative Role of Exercise. *Circulation Research*, *137*(2), 184–204. https://doi.org/10.1161/CIRCRESAHA.125.325532

Wu, H., & Ballantyne, C. M. (2020). Metabolic Inflammation and Insulin Resistance in Obesity. *Circulation Research*, *126*(11), 1549–1564. https://doi.org/10.1161/CIRCRESAHA.119.315896

Yang, Z., Scott, C. A., Mao, C., Tang, J., & Farmer, A. J. (2014). Resistance Exercise Versus Aerobic Exercise for Type 2 Diabetes: A Systematic Review and Meta-Analysis. *Sports Medicine*, *44*(4), 487–499. https://doi.org/10.1007/s40279-013-0128-8

Yunn, N.-O., Kim, J., Ryu, S. H., & Cho, Y. (2023). A stepwise activation model for the insulin receptor. *Experimental & Molecular Medicine*, *55*(10), 2147–2161. https://doi.org/10.1038/s12276-023-01101-1

Zhao, X., Huang, F., Sun, Y., & Li, L. (2025). Mechanisms of endurance and resistance exercise in type 2 diabetes mellitus: A Narrative review. *Biochemical and Biophysical Research Communications*, *761*, 151731. https://doi.org/10.1016/j.bbrc.2025.151731