Exercise and Muscle Lipid Content, Composition, and Localization: Influence on Muscle Insulin Sensitivity

Bryan C. Bergman[1] and Bret H. Goodpaster[2]

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Accumulation of lipid in skeletal muscle is thought to be related to the development of insulin resistance and type 2 diabetes. Initial work in this area focused on accumulation of intramuscular triglyceride; however, bioactive lipids such as diacylglycerols and sphingolipids are now thought to play an important role. Specific species of these lipids appear to be more negative toward insulin sensitivity than others. Adding another layer of complexity, localization of lipids within the cell appears to influence the relationship between these lipids and insulin sensitivity. This article summarizes how accumulation of total lipids, specific lipid species, and localization of lipids influence insulin sensitivity in humans. We then focus on how these aspects of muscle lipids are impacted by acute and chronic aerobic and resistance exercise training. By understanding how exercise alters specific species and localization of lipids, it may be possible to uncover specific lipids that most heavily impact insulin sensitivity.

Fatty Acid Metabolism, Intramuscular Triglycerides, and Insulin Resistance

Fatty acids can serve as a key fuel source for contracting and exercising muscle (1). Intramuscular triglycerides (IMTG) were first described by Denton and Randle (2) and soon thereafter were reported to be used during exercise (3). Contemporaneous studies by Randle et al. (4) reported that aberrant fatty acid metabolism was implicated in diminished glucose uptake and diabetes, and later studies collectively concluded that reduced capacity for fatty acid oxidation within skeletal muscle was implicated in excess IMTG accumulation (5,6), insulin resistance (7), and type 2 diabetes (8). This large body of work seemed to be consistent with initial studies performed in the 1990s linking IMTG content to insulin resistance and type 2 diabetes (9,10).

These early studies were, unknowingly, in apparent contrast in supporting both positive and negative roles for IMTG in health and disease. Moreover, the question of whether IMTG were harmful or beneficial was highlighted by the "athlete's paradox" (11), in which endurance-trained athletes have IMTG content similar to that of individuals with type 2 diabetes and yet are very insulin sensitive. Over the past two decades, numerous subsequent studies have attempted to define "good" and "bad" muscle lipids and to disentangle this conundrum. New ideas along with advances in muscle lipid composition and localization warrant an updated review on this topic. Gaps in knowledge and understudied areas are highlighted to focus future research efforts and bring more clarity to this complex area.

Diacylglycerol, Sphingolipids, Acylcarnitines, and Insulin Resistance

Model systems as well as human studies, aided by advances of mass spectrometry, have shifted the field beyond triglycerides to recognize that specific complex lipids within muscle may be more deleterious than others and are more likely implicated in mechanisms underlying insulin resistance (12–17). These other lipids associated with insulin resistance include diacylglycerol (DAG), sphingolipids, long-chain acyl-CoA (LCA-CoA), and acylcarnitines, as well as others (Fig. 1).

Muscle DAG accumulation has been implicated in insulin resistance, first in denervated rodent muscle (18), as well as following intralipid infusion in humans (19). DAG activate atypical PKC isoforms to decrease insulin signaling (20). In vitro studies showed that increased PKC activity results in decreased insulin-stimulated glucose uptake through phosphorylation of serine residues of IRS-1, including direct PKC phosphorylation of Ser^{1101} (21), enhanced c-Jun N-terminal kinase (JNK) and inhibitor of κB kinase (IKKB) phosphorylation of Ser^{307} (22), and increased

[1]University of Colorado Anschutz Medical Campus, Aurora, CO
[2]Translational Research Institute, AdventHealth, Orlando, FL
Corresponding author: Bryan C. Bergman, bryan.bergman@cuanschutz.edu

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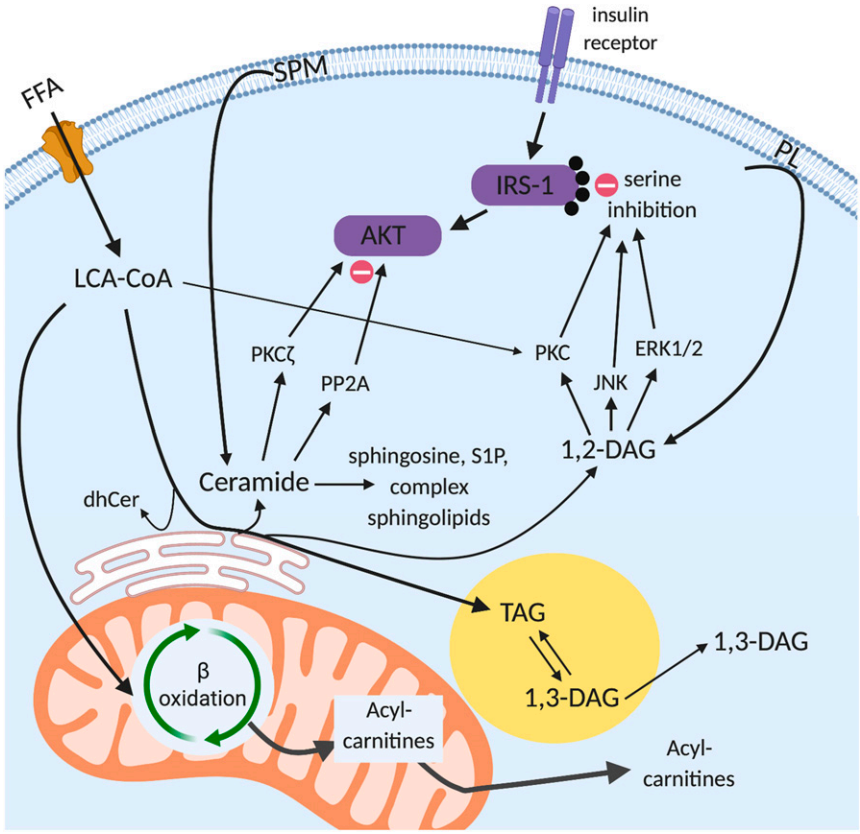

Figure 1—Potential mechanisms by which intramuscular lipids impact insulin sensitivity in skeletal muscle. AKT, protein kinase B; dhCer, dihydroceramide; FFA, free fatty acids; IRS-1, insulin receptor substrate-1; PKC, protein kinase C; PL, phospholipids; PP2A, protein phosphatase 2A; SPM, sphingomyelin; TAG, triacylglycerol.

p44/42 MAPK activity and Ser^{612} and Ser^{636} phosphorylation (23) resulting in decreased signaling through the phosphatidylinositol-3 kinase/AKT pathway (24). Sphingolipids have also been implicated in insulin resistance, with muscle ceramide accumulation first reported in obese insulin-resistant humans (25). In vitro studies revealed ceramides reputedly decrease insulin sensitivity by activating protein phosphatase 2A and PKCζ, which dephosphorylate AKT (26), and/or retain AKT in caveolin-enriched microdomains, which decreases insulin signaling (27). Dihydroceramides have recently been appreciated as bioactive molecules with the ability to impact cellular signaling through mechanisms distinct from ceramide, although whether they impact insulin sensitivity is unclear (28). Sphingosine may antagonize DAG-induced insulin resistance, as it has been shown to inhibit PKC and decrease DAG content in several cell types (29). Sphingosine can be phosphorylated to sphingosine-1-phosphate (S1P), which decreases ceramide content and promote increases insulin sensitivity in mice (30). However, the importance of sphingosine and S1P to muscle insulin sensitivity in humans is unclear, as muscle concentrations are not different (12,15,16,25) or greater (6) in insulin-resistant compared with insulin-sensitive individuals. Glucosylceramides and downstream gangliosides have been linked with insulin resistance in animal models (31), an effect that appears more potent in adipose tissue and liver compared with muscle (32). Similarly, lactosylceramides are also related to decreased insulin sensitivity in rodents (33). Other sphingolipids such as sphingomyelin may also be related to insulin resistance. However, sphingomyelin does not appear to directly impact insulin sensitivity in myotubes (34) but may be a pool from which ceramides are made in vivo (35). LCA-CoA accumulate and promote insulin resistance in muscle of rodents (36) and humans (37). In vitro studies show that LCA-CoA can activate PKC to decrease insulin signaling (38) and are also nuclear ligands (39).

Changes or differences in more polar lipids such as acylcarnitines are often linked with perturbed fatty acid oxidation and are associated with insulin resistance, and yet the exact mechanism by which they influence insulin sensitivity is not known (40). However, individuals with mutations in LCA-CoA dehydrogenase deficiency have significantly elevated acylcarnitines compared with age-matched control subjects yet have similar glucose tolerance, suggesting that a direct effect of acylcarnitines on insulin sensitivity is unlikely (41). There are many other polar lipids that may also impact insulin sensitivity that have received less attention in muscle,

including phosphatidic acid, lysophosphatidic acid, gangliosides, and ceramide-phosphate, among others.

Experimental alterations in muscle lipids have been reported to affect insulin sensitivity in lipid-induced insulin resistance in humans (19,42), although the response of DAG and sphingolipids to insulin-sensitizing lifestyle interventions is variable (15–17,43–46). Additionally, there are paradoxical studies in the literature dissociating DAG and sphingolipid content from insulin sensitivity in humans (47,48) and animal models (49,50). These conflicting reports highlighted that changes in lipid concentration do not consistently explain alterations in insulin sensitivity and/or that factors other than the total content of bioactive lipids play a role in decreasing insulin sensitivity. Later in this article, we will discuss studies using acute exercise and training to support a role for specific lipids within muscle in insulin resistance. However, other factors that may play a role in conflicting data are the difficulties in measuring muscle lipid content, the diverse techniques to measure lipid content, and improvements in methodologies such as mass spectrometry with greater breadth and sensitivity. These areas are not the topic of this article but have certainly played a role in shaping disparate results in the literature.

Specific Intramyocellular Lipid Isoforms and Species Likely Promote Insulin Resistance

The total amount or concentration of any class of lipid may be inadequate to explain function. Diversity of fatty acids in our diet as well as enzymatic synthesis, elongation, and desaturation leads to diversity in the acyl groups of all complex lipids. Different combinations of acyl groups result in various molecular species of lipids, each of which may have unique biological actions. For example, DAG is formed from two acyl chains esterified to a glycerol backbone resulting in many possible species. Further, there are three possible isomers of DAG based on the location of the two acyl chains on the three carbons of the glycerol backbone (1,2-, 1,3-, and 2,3-DAG) (51). Various DAG isomers and species are important because only 1,2-DAG are thought to activate PKC, and within 1,2-DAG isomers there is variable potency of each DAG species to activate PKC (20). Advances in mass spectrometry have facilitated measurement of specific species of lipids as they relate to insulin resistance. Data are mixed on whether specific molecular species of DAG uniquely induce insulin resistance, with some indication that di-saturated DAG species are more negative toward insulin resistance (13) and one report that di-unsaturated DAG are elevated in insulin-resistant muscle (44). Understanding species level detail is likely critical to understanding how these lipids can impact metabolic function and insulin sensitivity.

It is also important to look beyond canonical PKC signaling to interpret how DAG may impact metabolism. The molecular basis of DAG specificity for PKC isoform activation is due to DAG binding to a C1 domain to alter PKC structure promoting membrane insertion and activation (52). However, other proteins also contain a C1 domain and thus could alter cellular signaling. These other C1 domain–containing proteins include chimaerins, RasGRPs, MUNC13s, protein kinase D, and DAG kinase (53). Few studies have addressed non-PKC DAG targets, how they could impact insulin sensitivity, and how they change with acute and chronic exercise training.

Similar to DAG, there are many types of sphingolipids, each with a unique structure, of which ceramide is widely studied. Within each type of sphingolipid there are many possible species based on the composition of the acyl chains, each of which may have unique biological actions (54). Unlike in the DAG literature, there is consistent agreement that specific species of sphingolipids are associated with insulin resistance, as several reports have shown C16:0 and C18:0 ceramide species to be most potent for decreasing insulin sensitivity (12,55).

There are also diverse species of LCA-CoA in skeletal muscle; however, in obese humans, most all species of LCA-CoA accumulate in skeletal muscle (5). LCA-CoA decreased after insulin-sensitizing weight loss, with the decrease in C16:0 containing LCA-CoA most related to an increase in insulin sensitivity (56). LCA-CoA, specifically C16:0 species, may impair mitochondrial ADP transport, which may influence reactive oxygen species formation and insulin sensitivity (57). Therefore, C16:0 LCA-CoA may be particularly potent in promoting insulin resistance, but the mechanism responsible is not known.

These data collectively support the hypothesis that species-level data are critical to understanding how bioactive lipids impact cellular signaling and promoted decreased insulin sensitivity in skeletal muscle.

Localization of Intramuscular Lipids Influences Insulin Sensitivity

The realization that specific species of lipids are linked to decreased insulin sensitivity was an advance but still oversimplified the complex reality of lipids in muscle. Muscle lipid content is impacted by fiber type, with more lipid in type I compared with type II fibers. Further, lipids exist in many subcellular compartments and are constantly being trafficked between cellular compartments. Alterations in compartmentation and trafficking of lipids may reveal differences between groups and interventions that have been overlooked by previous studies. Emerging evidence shows that localization of triglycerides, DAG, and sphingolipids appears to play an important role in promoting decreased insulin sensitivity (35,42,58) (Fig. 2).

Triglyceride

IMTG storage is impacted by muscle fiber type. IMTG content is greater in type I compared with type II fibers except in individuals with type 2 diabetes, where IMTG content is more evenly split between type I and II fibers (59). IMTG is further compartmentalized in skeletal muscle, as transmission electron microscopy revealed subsarcolemmal IMTG was negatively related to insulin sensitivity in type II fibers, while intermyofibrillar IMTG was unrelated or positively related to

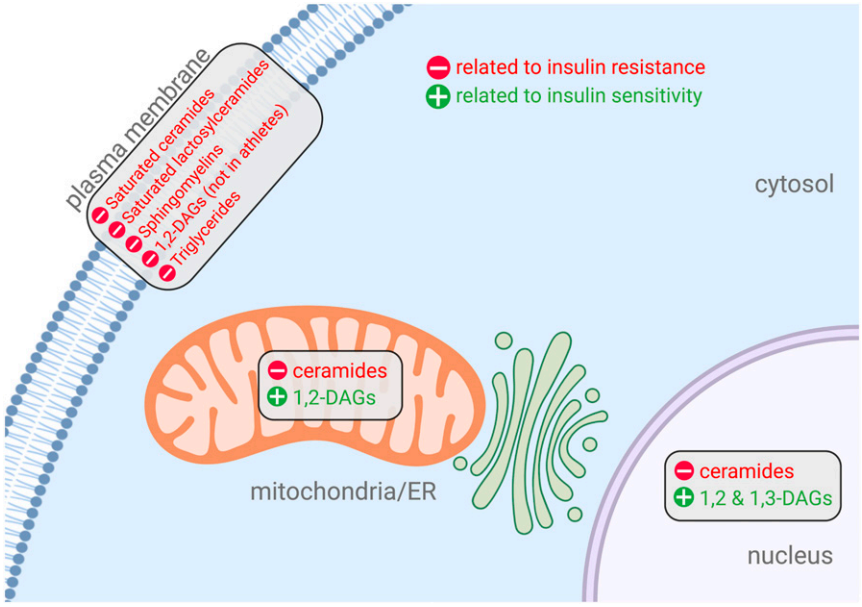

Figure 2—Relationships between lipid localization and insulin sensitivity in skeletal muscle in humans. Lipids in red, negatively related to insulin sensitivity; lipids in green, positively related to insulin sensitivity. Cytosolic lipids were not related to insulin sensitivity.

insulin sensitivity regardless of fiber type. Further, exercise training decreased subsarcolemmal IMTG distribution, which was associated with increased insulin sensitivity (60). The physical location of IMTG droplets relative to mitochondrial is likely important, as their close proximity may facilitate oxidation of IMTG (61).

DAG

Early work from the Bergman laboratory showed that DAG localized in skeletal muscle membranes were negatively related to insulin sensitivity, while DAG in the cytosolic compartment were unrelated to insulin sensitivity (13). These findings were repeated by others (42,58), and collectively these data pointed to the need for a more detailed understanding of how DAG compartmentation relates to insulin sensitivity in skeletal muscle. We recently reported on the distribution of DAG isomers and species in sarcolemmal, cytosolic, nuclear, and mitochondrial/endoplasmic reticulum (ER) compartments in skeletal muscle from individuals spanning the physiological spectrum of insulin sensitivity (35). We found that sarcolemmal 1,2-DAG accumulated in athletes, as well as obese individuals with and without type 2 diabetes relative to lean control subjects. This was unexpected and suggested that there is further subsarcolemmal localization of DAG in athletes that may explain similar DAG accumulation despite dichotomous sensitivity to insulin. 1,3-DAG isomers were not different in any compartment between groups. Unexpectedly, we found that mitochondrial/ER 1,2-DAG accumulated in athletes and lean individuals and was positively related to insulin sensitivity. More work is required to understand whether this accumulation represented mitochondrial 1,2-DAG accumulation that may be related to dense cristae packing found in athletes (62) or ER accumulation that reflects high rates of IMTG synthesis in athletes and insulin-sensitive individuals (63–66).

Sphingolipids

Ceramides have been known to be localized in various compartments in different cell types for many years (67). However, the relationship of localized sphingolipids to insulin sensitivity in human skeletal muscle was only recently appreciated. Chung et al. (68) showed that subsarcolemmal ceramides, specifically the C16:0 and C18:0 species, were negatively related to insulin sensitivity. We confirmed this finding and extended it to suggest that ceramides in sarcolemmal, mitochondrial/ER, and nuclear compartments were negatively related to insulin sensitivity (35). We also found that the relationship between saturated ceramides and decreased insulin sensitivity was strong, particularly for C18:0 ceramide. Sarcolemmal accumulation of sphingomyelin and lactosylceramides was also inversely related to insulin sensitivity. Therefore, accumulation of sphingolipids in any compartment appears to be related to insulin resistance.

Polar Lipids

Little is known regarding the subcellular compartmentation of acylcarnitines, sphingosine, ceramide-1-phosphate, gangliosides, phosphatidic acid, phospholipids, and LCA-CoA. Based on their function, acylcarnitines would be expected in the mitochondrial and the cytosolic compartments. Nuclear acyl-CoA and phospholipids are thought to be nuclear ligands influencing gene transcription (39). LCA-CoA are thought to be compartmentalized in distinct pools in skeletal muscle of

rodents, but analysis of concentration in specific locations in humans has not been performed (69).

It is unlikely that we will fully understand how muscle lipids impact diabetes risk or how they may be implicated in treating insulin resistance or diabetes until we understand the mechanisms of how specific species of lipids in specific cellular locations modify insulin sensitivity. Further, we need to embrace the complexity and interpret the full breadth of muscle lipids together in order to understand how these diverse pools of molecules collectively impact insulin sensitivity. Publications too frequently focus on individual lipids or lipid species in isolation as they relate to insulin resistance. It is alluring to try to simplify complexity, but we need to consider the minutia in order to progress understanding.

Now that we have provided some background for muscle lipids as they related to insulin resistance, we can dive into how exercise impacts muscle lipid content, species, and localization to influence insulin sensitivity. As exercise can be a powerful insulin-sensitizing intervention, it can also be a powerful tool to reveal and refute the potential roles of complex muscle lipids in insulin resistance.

Exercise and Muscle Lipids

Exercise is a cornerstone in lifestyle interventions to prevent diabetes (70) and a powerful tool to enhance insulin sensitivity. Evaluating how acute and chronic aerobic and resistance exercise impacts muscle lipid content, composition, and localization as it relates to insulin resistance may help reveal mechanisms for the insulin-sensitizing effects of exercise. Ultimately, understanding how exercise modifies muscle lipids to impact insulin sensitivity can also reveal how obesity, inactivity, and sedentary behavior promote muscle insulin resistance, prediabetes, and type 2 diabetes.

Acute Exercise, Insulin Sensitivity, and Muscle Lipids

Insulin Sensitivity Increases After Acute Aerobic and Resistance Exercise

Insulin sensitivity increases by 18–30% after an acute bout of aerobic exercise and persists for up to 48 h (71). The time course of this response indicates that only some of this increase can be attributed to glycogen depletion, as enhanced insulin sensitivity post-exercise takes 6 h to reach a peak despite persistent glycogen degradation throughout this period (72). Several groups have reported that muscles stimulated to contract acutely in vitro only show increased insulin sensitivity when contractions are performed in serum, suggesting that humoral factors in addition to glycogen depletion play an important role in insulin sensitization (73). These humoral factors are not known and highlight significant gaps in knowledge regarding acute exercise–induced insulin sensitization. Similar to aerobic exercise, resistance exercise increases insulin sensitivity even after one exercise session (74), but there is a paucity of mechanistic studies to explain these effects. The metabolic and physiologic stress with resistance exercise is different from that with aerobic exercise, and therefore it is likely that molecular mechanisms are quite different between these two exercise modalities.

Acute Aerobic Exercise Effects on Muscle Lipids

An acute bout of exercise is associated with an acute inflammatory response, which abates after several hours of recovery (75). Inflammation can drive formation of lipids such as sphingolipids, and thus an acute inflammatory response may impact the content of muscle lipids immediately after exercise. It would be expected that these inflammation-responsive lipids would then change during recovery. There are limited time course data on muscle lipids during recovery from exercise. Results from acute exercise studies should be interpreted with the understanding that muscle lipids could change significantly over the ensuring minutes and hours following a single bout of exercise.

The metabolic response to an acute bout of exercise will also influence degradation of muscle lipids. Exercise of longer compared with shorter duration will increase plasma free fatty acid concentration, which influences FFA uptake and the availability of LCA-CoA for bioactive lipid formation (76). IMTG utilization during exercise is influenced by circulating FFA content, with lower FFA concentration resulting in greater utilization of IMTG as a fuel source during exercise (77). The impact of circulating FFA concentration on other bioactive lipids is less clear but may impact skeletal muscle lipid content.

IMTG. Most studies report that an acute bout of aerobic exercise decreases IMCL content in athletes and lean individuals but not in obese individuals with or without type 2 diabetes (63,78) (Fig. 3). IMTG degradation during exercise occurs preferentially from type I compared with type II fibers (79,80). The utilization of IMTG is regulated by FFA availability, with decreased plasma FFA increasing the utilization of IMTG during exercise in both lean individuals and those with type 2 diabetes (77). In animal models, IMTG decreases after an acute bout of hindlimb contraction in obese but not lean rodents (81,82). It has also been reported that IMTG content increased after acute exercise (78) and that IMTG use occurs during recovery from exercise in humans (83), although there are also data showing no change in IMTG during recovery (84).

There is also evidence for sex-based differences in IMTG use during exercise. Women have greater IMTG stores compared with age- and BMI-matched men (85). Women also appear to have greater utilization of IMTG during exercise compared with men (85).

Acute exercise may also change IMTG localization, with enhanced mobilization of IMTG from subsarcolemmal compared with intermyofibrillar depots (86). Because subsarcolemmal IMTG is negatively related to insulin sensitivity, the mobilization of this depot by exercise could be a mechanism by which exercise is insulin sensitizing.

DAG. There are limited data on changes in muscle DAG and sphingolipids in response to acute exercise. We found that an acute bout of exercise did not change whole cell DAG concentration in athletes and obese individuals with

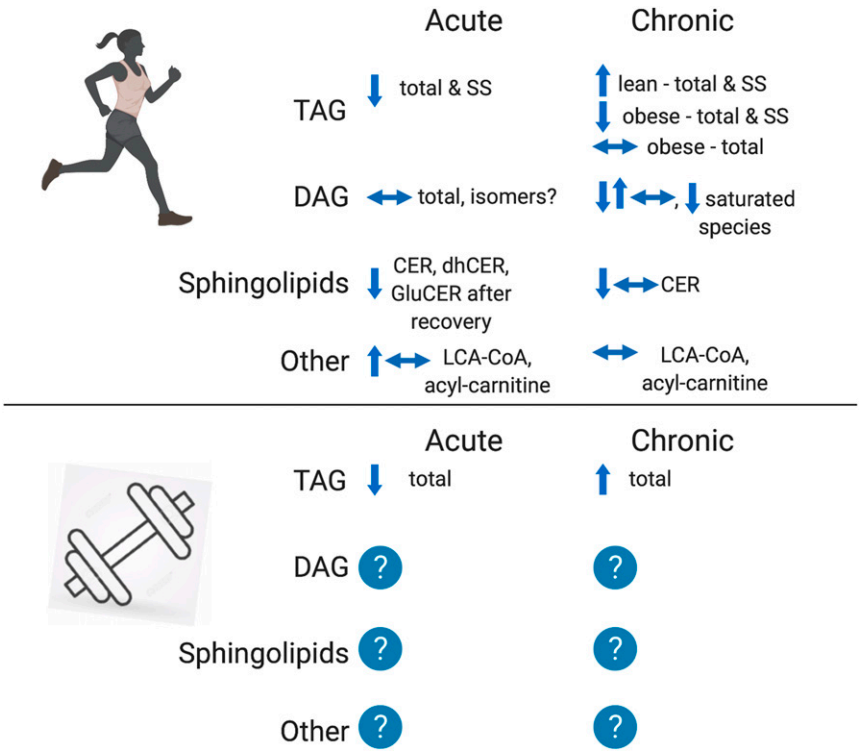

Figure 3—Impact of acute and chronic aerobic and resistance exercise training on content, species, and localization of lipids in skeletal muscle in humans. CER, ceramide; dhCER, dihydroceramide; GluCER, glucosylceramide; SS, subsarcolemmal; TAG, triglyceride.

and without type 2 diabetes (63). Similar data have been reported in rodents after an acute bout of exercise (87) and using an isolated contracting hindlimb model in lean and obese animals (81,82). Therefore, the total DAG pool appears stable during acute exercise. However, it is not clear whether acute exercise influences the relative proportion of DAG isomers. IMTG lipolysis during exercise liberates 1,3- and 2,3-DAG, which are not thought to activate PKC and impact insulin sensitivity. Therefore, IMTG utilization during exercise liberating 1,3- and 2,3-DAG isomers along with unchanged DAG concentration may indicate a decreased proportion of 1,2-DAG species. It not known whether a shift in DAG isomers post-exercise is yet another potential mechanism promoting insulin sensitization with acute exercise.

Sphingolipids. In humans, acute exercise increases muscle sphingosine, S1P, and ceramide in trained and untrained individuals (12,46). The immediate post-exercise increase may be due to an acute inflammatory response that drives ceramide synthesis, which abates after recovery (75,88). The activity of serine palmitoyltransferase, the rate-limiting step in ceramide biosynthesis, has been shown to increase with increasing duration of exercise in rodent skeletal muscle, which may also contribute to an acute increase in muscle ceramide content (89). After 2 h of recovery, sphingosine, S1P, and ceramide decrease to values equal or less than those of the rest in human muscle (12). Based on changes in mRNA expression, an increase in ceramide clearance in recovery appears to promote decreased ceramide content (12). Skeletal muscle sphingomyelin increased after an acute bout of exercise in untrained, but not trained, individuals, making it unlikely that sphingomyelin degradation to ceramide explains post-exercise ceramide accumulation (46). In an isolated contracting hindlimb animal model, acute exercise did not alter muscle ceramide content (90). However, in rodents acute exercise has been shown to decrease both ceramides and sphingomyelin (87) or increase ceramides after exhaustion (89). It is unclear why there appear to be differences in the effects of acute exercise on ceramide accumulation in humans and rodents. Dihydroceramides and glucosylceramides follow a pattern similar to that of ceramide, with an increase in content immediately post-exercise and decreasing to values similar to or less than rest after 2 h of exercise (12). These changes may also promote insulin sensitization after an acute bout of exercise (32).

Polar Lipids. LCA-CoA increased after an acute bout of contraction in an isolated hindlimb model in both lean and obese animals (81). Increased LCA-CoA occurred concomitantly with increased insulin sensitivity, suggesting that LCA-CoA does not always promote decreased insulin sensitivity, at least in rodents. The response to whole-body exercise is variable, with no change (91) or increased concentration (69) observed in rodents. Short-chain acylcarnitines increased after acute exercise in rodents, with no changes or an increase reported for short-, medium-, and long-chain

acylcarnitines in humans (82). Combined, these data suggest that the increase in insulin sensitivity from acute exercise is not likely to be explained by alterations in the content of LCA-CoA and acylcarnitines. The effect of acute exercise on the diverse milieu of polar signaling lipids in skeletal muscle is largely unknown.

Acute Resistance Exercise Effects on Muscle Lipids

IMTG. Similar to aerobic exercise, acute resistance exercise decreases muscle IMCL content in humans (92) (Fig. 3). The rate of utilization of IMTG during resistance exercise appears to be in part dictated by the starting concentration such that greater IMTG content results in increased rates of IMTG degradation (92). After 2 h of recovery after resistance exercise, IMCL content was unchanged (93) or had returned to basal levels (94).

DAG, Sphingolipids, and Polar Lipids. Alterations in bioactive lipids such as DAG, sphingolipids, and other polar lipids such as acylcarnitines in response to acute resistance exercise are unknown.

While the effect of acute resistance exercise on muscle lipid content is not well studied, there is evidence that aging and muscle lipids may interact to influence the anabolic response to exercise. Aging is associated with increased content of muscle lipids as well as attenuated anabolic response to resistance exercise (95). It has also been shown that ceramide accumulation is required for anabolic resistance associated with inflammatory cytokine exposure in myotubes (88). Therefore, it is possible that muscle sphingolipid content influences the ability of aging muscle to adapt to resistance exercise, which promotes the development of sarcopenia.

Chronic Exercise, Insulin Sensitivity, and Muscle Lipids

Insulin Sensitivity Increases After Chronic Aerobic and Resistance Exercise

Chronic endurance exercise training is the cornerstone of lifestyle interventions and consistently increases insulin sensitivity (96). The training effect is not due solely to glycogen depletion, as insulin sensitization remains long after glycogen is replaced following the last exercise bout. There are other mechanisms responsible for increased insulin sensitivity including increased GLUT4 content, capillary density, and mitochondrial content among others (97). Our studies (12,13,17,44,45,64), and those of many others (43,98), suggest that exercise-induced improvement in insulin sensitivity may be related to changes in intramyocellular lipids. Similar to aerobic exercise, chronic resistance training is also effective at increasing insulin sensitivity in humans (74,99). The data are mixed regarding whether aerobic or resistance exercise is more effective than the other to increase insulin sensitivity (100), and there is a paucity of detailed mechanistic studies with resistance training. Some investigators concluded that the increase in muscle mass was responsible for greater glucose uptake after resistance training (101), while some, but not all, found that resistance training increased mitochondrial density, oxidative capacity, and GLUT4 content, which may influence insulin sensitivity (102). The metabolic and physiologic stresses with resistance exercise are different from those with aerobic exercise, and therefore it is likely that molecular mechanisms for insulin sensitization as well as changes in muscle lipid content and composition are very different between these two exercise modalities.

Chronic Aerobic Exercise Alters Muscle Lipid Content

IMTG. The response of IMTG to chronic aerobic exercise training is variable. In lean individuals, IMTG content increases in response to aerobic exercise training (61), although no changes have also been reported (103). However, in obese individuals with and without type 2 diabetes, after chronic aerobic exercise training, IMTG has been shown to increase (45), decrease (104), or not change (43). When exercise training is combined with energy restriction, most studies have found that IMTG content remains unchanged in obese individuals (105). This is likely due to the counterbalancing effect of energy restriction and weight loss to decrease IMTG and exercise training to increase IMTG (10,45). The variability in the response of IMTG to insulin-sensitizing chronic endurance exercise training reinforces the idea that IMTG itself does not impair insulin sensitivity.

Insulin-sensitizing exercise training has also been shown to alter IMTG localization. After chronic aerobic training, there was decreased subsarcolemmal and increased intermyofibrillar triglyceride size and number, as well as contact with the mitochondrial reticulum, in lean and obese men and women (60,61). Therefore, changes in IMTG localization, even when there is no change in total content, may play a role in increased insulin sensitivity.

DAG. Total DAG content has been reported to be higher in endurance-trained athletes, creating another "athlete's paradox" with DAG and insulin resistance. Chronic aerobic exercise training, however, has been reported to decrease muscle DAG content in overweight and obese individuals (17,43–45). Endurance training may also alter the composition of DAG to be less saturated (43), which may impact insulin sensitivity (13). However, no change in DAG content has also been reported in lean and obese men and women following insulin-sensitizing exercise training without weight loss (60) and in obese men and women after combined gastric bypass surgery and exercise training (16). These data indicate that, while chronic endurance exercise training can decrease whole cell DAG concentration and composition, these changes are not required for enhanced insulin sensitivity. One way to interpret these data is that changes in whole cell muscle DAG content with exercise training overlook important aspects of DAG metabolism that influence insulin sensitivity. There could be alterations in DAG isomers, species, and/or localization after chronic training that have gone unnoticed. This level of detail may help uncover specific species and compartments altered by exercise that impact insulin sensitivity. Thus, there is a need for intervention studies with

detailed lipidomic analysis to elucidate interrelationships of specific DAG isomers, species, and localization on changes in insulin sensitivity.

Sphingolipids. Chronic aerobic exercise training with or without weight loss typically decreases muscle ceramide content (16,17,43,45,98). However, unchanged ceramide content has also been reported following exercise training with (106) and without (60) weight loss. Muscle sphingosine and S1P content are unchanged in trained compared with untrained individuals (12), as well as following exercise training with or without weight loss (16,17). The response of other sphingolipids to chronic exercise training is less well defined. Sphingomyelin decreased after 6 weeks of exercise training in rats (107). Similar to DAG, ceramides can also be uncoupled from insulin sensitivity. These data suggest that total ceramide content is not always the main factor in insulin resistance and raises questions about the importance of specific species and/or localization that may play an important role in insulin desensitization as reported by others (13,35,55).

Polar Lipids. Longitudinal studies investigating changes in polar lipids including LCA-CoA are scarce. However, there does not appear to be a change in LCA-CoA after chronic exercise training without weight loss in overweight or obese individuals with and without type 2 diabetes (104). It is possible that specific species of LCA-CoA could be modified by exercise training; however, species-specific information has not yet been published. Therefore, changes in LCA-CoA content are unlikely to explain increased insulin sensitivity after chronic aerobic exercise training. Changes in muscle acylcarnitine content are also not likely to explain the increases in insulin sensitivity, as acylcarnitines are increased after chronic exercise training (108). Detailed studies investigating changes in the breadth of polar lipids after exercise training interventions are needed.

Chronic Resistance Exercise Alters Muscle Lipid Content

IMTG. Similar to aerobic exercise, insulin-sensitizing chronic resistance exercise training increased IMTG content (99). This is yet another example of decoupling IMTG content to insulin sensitivity.

DAG, Sphingolipids, and Polar Lipids. The impact of chronic resistance training on DAG, sphingolipids, and polar lipid content and composition has received little attention. Resistance training alone does not alter muscle acylcarnitine content, suggesting that increases in insulin sensitivity cannot be explained by these lipids (108). Little is known regarding how resistance training impacts muscle lipids.

Areas Needing Further Research

Human clinical investigations using acute and chronic exercise experiments have promoted a better understanding of how muscle lipids may be implicated in insulin resistance and type 2 diabetes. While these studies rarely identify precise molecular mechanisms, they can, through reduction, eliminate or refute mechanism or causes. It should also be emphasized that skeletal muscle insulin resistance is complex and multifaceted and likely has many causes and, thus, has many potential remedies.

While this review was focused mostly on triglyceride, DAG, and sphingolipids, we acknowledge that the effects of exercise on phospholipids are also likely important. There are many classes of phospholipids, each with individual species, as well as lysophospholipids and oxidized phospholipids, each of which can play roles in cell signaling. A separate work on the effects of exercise on phospholipid metabolism is warranted.

Several areas of investigation will further our understanding of how muscle lipids play a role in insulin resistance and metabolic diseases. First, triglycerides are frequently measured as one entity, yet we know that there are hundreds of species that make up "triglyceride" based on combinatorial probability for variable acyl side chains on each of the three carbons of the glycerol backbone. Are there specific species of triglyceride that promote decreased insulin sensitivity? How does exercise training impact specific triglyceride species? Does the localization of these species change with exercise? Second, most of this review was focused on the role of "static" neutral lipids within muscle, since so few studies have investigated the dynamics or turnover of muscle lipids. A popular hypothesis is that exercise increases IMTG turnover, which promotes insulin sensitivity by acting as a sink into which LCA-CoA can be stored (109). High rates of IMTG turnover and LCA-CoA esterification are thought to prevent formation of bioactive lipids promoting decreased insulin sensitivity. This has been shown experimentally in both humans (110) and animals (111), and cross-sectional comparisons also show a positive relationship between IMTG synthesis rates and insulin sensitivity (63,65,66).

While the analysis of quantity and localization of muscle lipids will continue to be critical, additional interrogation of the molecular pathways that affect muscle lipids and concomitant insulin sensitivity is imperative. It will be important to understand whether acute and chronic exercise impact muscle epigenetics that regulate insulin sensitization, as well as alterations in the muscle phosphoproteome, e.g., FABP, acyl-CoA BP, and CERT, following aerobic and resistance exercise that may impact muscle lipid content, localization, and insulin sensitivity. The Molecular Transducers of Physical Activity Consortium (MoTrPAC) exercise training study will be particularly useful to reveal molecular regulation of muscle lipids after acute and chronic aerobic and resistance exercise. Few studies have addressed non-PKC DAG targets and how they could impact insulin sensitivity. These types of studies, collectively, if performed in larger numbers of subjects, could yield information about the variation in responses to insulin-sensitizing interventions such as exercise (most studies to date have been too small to adequately determine biological response variation). Does the degree of change in muscle lipid species and/or localization influence the response variation for the change in insulin sensitivity due to exercise? It will be critical to better understand how exercise training, with or without weight loss,

influences muscle lipid localization and the change in insulin sensitivity.

Conclusions

Accumulation of muscle lipids, specifically DAG and sphingolipids, is related to decreased insulin sensitivity in humans. Specific species of lipids appear to play more deleterious roles, and more recent data indicate that localization of these lipid species impacts their ability to induce or worsen insulin resistance. Both aerobic and resistance exercise improve insulin sensitivity, and each of these modalities appears to impact accumulation of lipids in muscle. However, there is much to learn regarding how acute and chronic exercise training impacts the content and localization of specific lipid species linked to insulin resistance. By understanding how exercise alters specific species and isomers of bioactive lipids, and how exercise changes localization of these lipids, it may be possible to uncover specific lipids that most heavily influence insulin sensitivity. Therefore, exercise will continue to be a powerful experimental tool to elucidate the complex relationships between muscle lipids and insulin resistance.

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