The Effect of Exercise and Nutrition on Intramuscular Fat Metabolism and Insulin Sensitivity

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Annu. Rev. Nutr. 2010. 30:13–34

First published online as a Review in Advance on April 7, 2010

The *Annual Review of Nutrition* is online at nutr.annualreviews.org

This article's doi: 10.1146/annurev.nutr.012809.104817

0199-9885/10/0821-0013$20.00

Key Words

intramuscular triglyceride (IMTG), intramyocellular lipid (IMCL), triglyceride synthesis, lipolysis, skeletal muscle, diet

Abstract

Intramuscular triacylglycerol (IMTG) is both a dynamic fat-storage depot that can expand during periods of elevated lipid availability and a fatty acid source that can be utilized during periods of increased energy expenditure in active individuals. Although many studies have investigated the lifestyle determinants of IMTG content, the results are far from consistent, and studies attempting to unravel the mechanisms behind IMTG metabolism are in their infancy. The limited evidence available suggests that the enzymes responsible for skeletal muscle lipolysis and IMTG synthesis play an important role in determining the fate of fatty acids and therefore the concentration of lipid metabolites and insulin sensitivity of skeletal muscle. This review provides a summary of current knowledge on the effects of acute and chronic exercise as well as energy intake and macronutrient composition of the diet upon the metabolism of IMTG and the implications for metabolic health.

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TG: triacylglycerol

IMTG: intramuscular triacylglycerol

LCFA-CoA: long-chain fatty acyl-CoA

DAG: diacylglycerol

INTRODUCTION

Fat is the main endogenous energy store within the human body and is primarily stored as triacylglycerol (TG). The majority of intracellular TGs are incorporated into discrete lipid droplets that are present in virtually all cell types and are composed of a core of neutral lipids (TGs and cholesterol esters) and surrounded by a phospholipid monolayer (81). In addition, a number of proteins are embedded into the outer membrane of the lipid droplets (141); the exact function of these proteins is yet to be determined. The vast majority of TG in the human body is stored within subcutaneous and visceral adipose tissue, with smaller amounts present in skeletal muscle fibers (intramuscular triacylglycerol; IMTG). The content of IMTG is two- to threefold greater in oxidative type 1 muscle fibers, which also exhibit a greater capacity for fat oxidation. Although the IMTG content makes up only a fraction (~1% to 2%) of the total fat stores within the body (120), this fuel store has generated great scientific interest over the past 10 to 15 years. Skeletal muscle lipid droplets are metabolically active and provide fatty acid substrate for oxidation, especially during periods of increased energy expenditure; in addition, they play roles in circulatory and intramuscular lipid homeostasis and the generation of intracellular signals. Elevations in IMTG content have also been linked to the development of metabolic diseases associated with obesity, such as insulin resistance and type 2 diabetes (82, 86).

More than a decade ago it was reported that the IMTG content within human muscle biopsies was inversely associated with insulin sensitivity (82, 86). It was postulated that the accumulation of fat in skeletal muscle was related to the inhibition of insulin action, although the mechanism of action was not known at that time. Interest was further stimulated by the paradoxical findings that accumulation of IMTG is an adaptation to chronic endurance exercise, as highly trained athletes exhibit similar if not greater concentrations of IMTG than those of sedentary obese or obese type 2 diabetics (37, 123).

It is now believed that increased concentrations of intramuscular lipid metabolites [long-chain fatty acyl-CoA (LCFA-CoA), diacylglycerol (DAG), and ceramide] are responsible for the impairment in insulin action rather than the inert IMTG pool located within lipid

droplets. LCFA-CoA and DAG activate PKC, which appears to induce impairments in insulin signaling through serine phosphorylation of the insulin receptor substrate-1. Alternatively, ceramide inhibits the insulin-signaling pathway downstream of PI3 kinase through the activation of protein phosphatase 2A, which dephosphorylates and inactivates Akt/PKB (for reviews, see 52, 96, 104). Although the evidence indicates that the IMTG content per se has no effect upon insulin sensitivity, a high turnover rate of the IMTG pool has been proposed to reduce the intracellular concentration of lipid metabolites (78) and therefore have a positive impact upon insulin sensitivity of skeletal muscle. The IMTG pool can provide a source of insulin resistance-inducing lipid metabolites and also provide a sink for lipid metabolites, thus protecting against insulin resistance. Therefore, the enzymes and mechanisms regulating the turnover (synthesis and breakdown) of the IMTG pool as well as oxidation of intracellular fatty acids are important in the maintenance of insulin sensitivity in skeletal muscle. The aim of this review is to describe the effects of acute and chronic exercise and of diet (energy intake and macronutrient composition) upon IMTG metabolism and insulin sensitivity.

INTRAMUSCULAR TRIACYLGLYCEROL UTILIZATION DURING EXERCISE

Acute Exercise and IMTG Oxidation

The IMTG pool is now considered by many to provide an important source of energy during endurance exercise in trained individuals in the fasted state (61, 120, 134). For example, van Loon et al. (123) demonstrated ~60% reduction in IMTG content in type 1 muscle fibers following two hours of cycling exercise performed at 60% maximum oxygen uptake ($VO_{2\,max}$). The specific depletion of IMTG in type 1 muscle fibers is a consistent finding and is in line with the greater capacity for IMTG storage and fatty acid oxidation in these fibers. The use of IMTG as a fuel source does not appear to be limited to endurance exercise either, as several studies have also demonstrated a significant reduction in IMTG content after a single bout of resistance exercise (31, 44, 64).

VO_{2max}: maximum oxygen uptake

The utilization of IMTG as a fatty acid source for β-oxidation and ultimately adenosine triphosphate resynthesis during exercise has proved a controversial issue. Over the past 30 years, IMTG content has been measured using numerous methodologies, including indirect estimates via tracer methodology, noninvasive measurements using magnetic resonance spectroscopy (MRS), and direct measurements from muscle biopsies using biochemical extraction techniques as well as immunofluorescence and electron microscopy imaging techniques. There are a number of inconsistencies in the literature, which have been reviewed previously (120, 134) and appear to be largely explained by methodological limitations. Studies using the biochemical TG extraction have shown significant decreases or no change following a single bout of exercise in both trained and untrained individuals [see previous reviews (61, 120) for individual references]. The majority of studies measuring IMTG noninvasively using MRS (61, 111, 113, 120) and (immuno)histochemically with oil red O staining of neutral lipids (23, 24, 110, 111, 123) have shown significant reductions in IMTG content after endurance exercise in lean, physically active individuals.

Because skeletal muscle is a heterogenous tissue, it is important to evaluate fiber type differences in IMTG content and also to discount potential contamination from extramuscular lipid sources. Histochemical staining of IMTG combined with immunofluorescence detection of muscle fiber type allows the visualization of individual lipid droplets in type 1 and type 2 muscle fibers. The use of image-analysis software allows quantification of the area fraction of the muscle fibers positively stained for IMTG and is the common method used to quantify IMTG content (37, 103, 106, 122, 123).

FA: fatty acid

This method has unique advantages as it provides information on fiber type differences and discounts the lipid contained between the muscle fibers from the IMTG estimate. It is important to note that this method cannot give true IMTG concentration measures owing to the semi-quantitative nature of the method, with the results being affected by the image capture settings. Therefore, this technique should only be used to compare IMTG changes within individual studies using exactly the same equipment and image capture settings, preferentially with comparisons made on a single microscope slide. A number of studies have utilized such methods to investigate IMTG content in response to exercise (23, 24, 110, 111, 123) and have demonstrated ~50%–70% depletion of IMTG during endurance exercise of two- to three-hour duration. Higher-resolution confocal microscopy allows the analysis of lipid droplet number and size (**Figure 1**). Furthermore, the visualization of IMTG also enables the collection of additional information such as lipid droplet morphology and subcellular localization and colocalization with enzymes controlling lipid metabolism (102, 103), which may all prove important in understanding the mechanisms of IMTG metabolism and its link with the development of insulin resistance (10, 45, 105). Therefore, we consider the immunofluorescence staining of IMTG to be the most informative method currently available to investigate changes in IMTG concentrations in response to exercise and dietary interventions and in the (patho)physiology of disease.

Although there is convincing evidence that the IMTG pool is depleted during prolonged exercise, this depletion appears to be restricted to lean active individuals. Numerous studies have shown that obesity is related to impairments in oxidative capacity and fatty acid oxidation (7, 8, 51, 58). Furthermore, indirect estimates of IMTG oxidation using ^{13}C-labeled palmitate suggest that IMTG oxidation is also very low during exercise in lean sedentary, obese, and obese type 2 diabetic individuals (7, 98, 120). Furthermore, one study failed to observe reductions in IMTG content in type 1 fibers after one hour of cycling in type 2 diabetics (124). In the same study, pharmaceutical lowering of plasma fatty acids by inhibiting adipose tissue lipolysis with Acipimox alleviated the impairment in IMTG oxidation. This indicates that a high rate of adipose tissue lipolysis in obese type 2 diabetics is related to an impairment in IMTG oxidation (124).

Rates of whole body fat oxidation and IMTG utilization are determined by factors such as diet, exercise intensity, exercise duration, and fitness. Maximal rates of fat oxidation occur at moderate exercise intensities (~60% $VO_{2\,max}$) (1). Results from isotopically labeled tracer studies estimate that the oxidation of TG sources accounts for 50% of fat oxidation at moderate exercise intensities (121). Exercise at a higher intensity is coupled with a substantial decline in total fat oxidation, which is derived from a reduction in both plasma and TG-derived fatty acids. Prolonged endurance exercise of moderate-low intensity is related to a progressive increase in fat oxidation over time, and significant declines in IMTG concentration have been observed after exercise duration from 2 to 7 hours. Although net declines in IMTG have been observed after very prolonged exercise, the utilization of IMTG appears to be limited to the first few hours of exercise. For example, Watt et al. (132) measured a significant decline in IMTG after two hours of moderate-intensity exercise (~57% VO_{2max}) but failed to observe further changes in IMTG content after four hours of exercise. It has been speculated that the gradual elevation in adipose tissue lipolysis during prolonged exercise inhibits intramuscular lipolysis and IMTG utilization. This appears to be confirmed by studies that have reduced plasma fatty acid (FA) concentrations through inhibition of adipose tissue lipolysis and observed increased utilization of IMTG (126, 137). It is possible that significant IMTG depletion in type 1 muscle fibers also contributes to the reductions in IMTG oxidation observed during prolonged exercise (123). The effects of diet and training on IMTG oxidation are discussed later in the review.

Feeding and IMTG Oxidation During Exercise

The interaction of fat and carbohydrate oxidation during exercise depends on the feeding status. It is common practice to consume carbohydrate during prolonged endurance exercise as a means to maintain plasma glucose concentrations and rates of carbohydrate oxidation (18). Such feeding strategies have been shown to improve exercise capacity and exercise performance (54). However, the effect of carbohydrate feeding during exercise on IMTG utilization is somewhat unclear. It has been shown that ingesting a 6.4% glucose drink before and during two hours of moderate-intensity exercise blunts hormone-sensitive lipase (HSL) activity in contracting skeletal muscle (138). However, the reduction in HSL activity was not associated with a reduction in IMTG oxidation (138). On the other hand, elevations in plasma insulin in response to carbohydrate feeding also inhibit adipose tissue lipolysis, reduce plasma FA concentrations, and suppress FA uptake in skeletal muscle (110, 138). Therefore, insulin-induced inhibition of adipose tissue lipolysis could lead to an increase in IMTG utilization in line with the increase in IMTG utilization seen after pharmacological suppression of adipose tissue lipolysis by Acipimox (124, 126). So far the results from a limited number of studies have been contradictory (24, 110). The composition of pre-exercise meals does appear to influence IMTG utilization during exercise. The use of IMTG has been shown to increase ~twofold when high-GI-index meals are eaten the day preceding exercise (113, 119). A more detailed description of the effect of nutrition and postexercise feeding follows in later sections of this review.

Skeletal muscle lipolysis and TG synthesis occur simultaneously at rest and during exercise; therefore, the IMTG content is determined by the balance between synthesis and breakdown. At rest, IMTG concentrations are unchanged, as synthesis and lipolysis occur at the same rate. IMTG concentrations only change when there is an imbalance between lipolysis (during exercise) and synthesis (after exercise and/or after feeding).

REGULATION OF SKELETAL MUSCLE LIPOLYSIS

The hydrolysis of IMTG requires enzymatic activation of lipases, which are regulated through a number of mechanisms (55). Skeletal muscle lipolysis is stimulated independently by muscle contractions and adrenaline. For a number of years, HSL was considered the major lipolytic enzyme in skeletal muscle; however, recent advances have shown an important role for other lipolytic enzymes and possibly for associated lipid droplet (LD) proteins that regulate the activity of lipases in skeletal muscle.

HSL: hormone-sensitive lipase

LD: lipid droplet

Hormone-Sensitive Lipase

Skeletal muscle expresses HSL protein (66) in greater concentrations in type 1 than in type 2 muscle fibers (68), which is in line with the observation that type 1 fibers exhibit higher IMTG oxidation rates during exercise (123). An increase in HSL activity has been shown to occur very quickly in response to exercise in humans (135). Immunoinhibition of HSL prevents the increase in lipolysis when rat muscle is electrically stimulated in vitro (67) or after 60 min of cycling at 70% VO_{2max} in man (139); therefore, HSL was considered to be the major lipolytic enzyme in skeletal muscle during exercise. The increase in rat muscle HSL activity in vitro is mediated by both adrenaline and muscle contractions, and these effects appear additive (65), suggesting that HSL activation during exercise occurs through a number of mechanisms. HSL has at least five serine phosphorylation sites (92, 93, 136), and adrenaline-stimulated (via protein kinase A) phosphorylation of these serine phosphorylation sites occurs during exercise in human skeletal muscle, along with an increase in HSL activity (93, 136).

Translocation of HSL to the LD has been demonstrated in adipocytes in vitro (28, 49). Furthermore, when rat muscle was either electrically stimulated or incubated with adrenaline,

ATGL: adipose triglyceride lipase

HSL translocation from microsomal stores to the LD was observed in isolated muscle fibers (88). Therefore, it appears that the subcellular redistribution of HSL to the LDs upon activation by muscle contraction or adrenergic stimulation is a crucial step in lipolysis. In adipose tissue, the translocation of HSL to the lipid droplets requires the phosphorylation of the lipid droplet protein perilipin, a protein coating the surface of adipocyte lipid droplets (114). Although perilipin is not present in skeletal muscle (41), other related LD-associated proteins are expressed (e.g., ADRP/adipophilin, TIP47, S3-12, and OXPAT; 76). HSL does translocate to the LDs containing ADRP and TIP47 when rat muscle fibers are adrenaline stimulated or contracted; however, their exact role in skeletal muscle fat metabolism and/or lipolysis has not been determined (88). Translocation of HSL to LDs has not yet been observed in human skeletal muscle in response to exercise.

Insulin has a potent antilipolytic effect in adipose tissue; however, the hormonal regulation of lipolysis in skeletal muscle is less clear (55). Glucose ingestion during exercise blunts the increase in HSL activity, possibly through elevations in circulating insulin and suppressed adrenaline (138). Somewhat surprisingly, insulin-mediated inhibition of in vivo muscle lipolysis is not apparent in all studies (e.g., 77), and sparing of IMTG oxidation with glucose ingestion has been shown in some but not all studies (22, 24, 110, 138). Skeletal muscle lipolysis appears to be regulated by the rate of adipose tissue lipolysis and potentially via elevated concentrations of plasma FA and intracellular concentrations of LCFA-CoA. HSL activity was reduced after 120 minutes of exercise despite elevations in circulating adrenaline and low levels of plasma insulin (133). The decrease in HSL activity at 120 minutes mirrors the reduced oxidation of IMTG after two hours of exercise (132). The reduction in HSL activity was associated with increased plasma FA concentrations and increased intracellular LCFA-CoA concentrations (133). Thus, it has been suggested that muscle lipolysis may be regulated to a higher degree by substrate supply and therefore fatty acid availability rather than insulin (55). This regulatory mechanism may also provide an explanation as to why no reductions in IMTG content were observed after one hour of exercise in type 2 diabetics (124). LCFA-CoAs are known to accumulate in the muscle of sedentary obese individuals (29) and in response to artificial elevations in plasma free fatty acid concentrations (intralipid/heparin infusions) (142). Therefore, elevations in intramuscular LCFA-CoA concentrations in obese insulin-resistant individuals may also be responsible for the impairment in the oxidation of IMTG due to the inhibition of skeletal muscle lipolysis. Although there is little information regarding the lipolytic activity of skeletal muscle in obese, insulin-resistant individuals, one study has demonstrated impaired adrenergic stimulation of muscle lipolysis in obese individuals (6).

Adipose Triglyceride Lipase

Although inhibition of HSL abolishes the majority of muscle TG-lipase activity in response to muscle contractions (67), at rest, between 40% and 80% of TG-lipase activity remains following HSL inhibition (2, 67, 139). Furthermore, basal TG-hydrolase activity is unaltered in HSL knockout mice, suggesting that HSL is not solely responsible for TG hydrolysis at rest (42). Interestingly, HSL knockout mice accumulate diacylgylcerol; HSL is therefore considered to be primarily responsible for the hydrolysis of DAG to monoacylglycerol (MAG), and another enzyme is responsible for the hydrolysis of TG (42). Adipose triglyceride lipase (ATGL) was first detected in adipose tissue and is primarily responsible for the hydrolysis of TG to DAG (144). Skeletal muscle expresses ATGL mRNA (144), and more recently it has been shown to contain ATGL protein with higher concentrations in type 1 than type 2 muscle fibers (56). The discovery of ATGL and potentially more lipases in skeletal muscle may explain the discrepancies between HSL activation and IMTG breakdown during exercise (138, 139).

Relatively little is known about the regulation of ATGL in skeletal muscle. In adipose tissue, the activation of ATGL is dependent on stimulation indirectly via PKA (144), which requires interaction of ATGL with its activator CGI-58. It is believed that perilipin phosphorylation releases CGI-58, which allows the activation of ATGL (39). CGI-58 is also expressed in skeletal muscle and upregulates ATGL activity in human skeletal muscle (2). The importance of CGI-58 in the activation of ATGL has been demonstrated in individuals with a CGI-58 mutation who are characterized by TG accumulation in many tissues, including skeletal muscle (15). In adipose tissue, ATGL activity increases in response to PKA (adrenaline) stimulation, but little is known about the hormonal regulation of ATGL in skeletal muscle.

The activation of HSL and ATGL are clearly important determinants of IMTG breakdown and therefore oxidation during exercise. In addition, the activity of these enzymes will ultimately influence the concentration of intracellular lipid metabolites and insulin sensitivity. For example, ATGL knockout mice demonstrate elevated IMTG concentrations but also improvements in insulin-mediated glucose transport in muscle as well as increased insulin-stimulated phosphorylation of insulin receptor substrate 1 and Akt and of PI3 kinase activity (60). It has recently been shown that the ratio of TAG hydrolysis/DAG hydrolysis is greater in insulin-resistant individuals (79). This led to the hypothesis that excessive TAG hydrolysis could explain the accumulation of DAGs and ceramide and therefore the development of insulin resistance in obese individuals.

REGULATION OF INTRAMUSCULAR TRIACYLGLYCEROL SYNTHESIS

IMTGs are synthesized by the sequential addition of three fatty acids to a glycerol-3-phosphate backbone (**Figure 1**). Here we give only a brief description; for more detailed recent reviews, see References 20, 115, and 140. The esterification of the first LCFA-CoA to glycerol-3-phosphate is the first committed step of IMTG synthesis and is mediated through the enzyme glycerol-3-phosphate acyltransferase (GPAT). Four isomers of this enzyme are present in skeletal muscle. GPAT1 and GPAT2 are present on the mitochondrial membrane and therefore are also named mitochondrial GPAT (mtGPAT). GPAT3 and GPAT4 are localized to the endoplasmic reticulum (for a recent review, see 115). The remainder of the TG synthesis steps take place in the endoplasmic reticulum (20, 115). The second acylation step in the IMTG synthesis pathway is catalyzed by the acylglycerol-3-phosphate acyltransferase (AGPAT) enzymes. In skeletal muscle, DAG is formed following the removal of a phosphate group by the phosphatidate phosphatase-1 enzyme, lipin1, and is the final precursor to the synthesis of IMTG. Diacylglycerol acyltransferase (DGAT) is responsible for the final step of IMTG synthesis, which involves the esterification of the third LCFA-CoA to diacylglycerol to form triacylglycerol. The newly formed TGs are incorporated into LDs (130). Oxidative fibers have a higher rate of IMTG synthesis at rest (13), and we have shown that the content of TG synthesizing enzymes is also higher in type 1 muscle fibers than in type 2 fibers in humans (**Figure 2**, J. Clark, C.S. Shaw, & A.J.M. Wagenmakers, unpublished observations). This is expected, given the higher IMTG content in type 1 muscle fibers.

Interestingly, the mitochondrial GPAT enzymes are present on the outer mitochondrial membrane, and in the liver they exhibit a reciprocal relationship with carnitine palmitoyltransferase I (CPT1; the rate-limiting enzyme of fatty transport across the mitochondrial membrane). When GPAT1 is overexpressed, FA oxidation is suppressed (72), whereas in GPAT1 knockout mice, fatty acid oxidation increases (43). Because CPT1 and GPAT1 appear to compete for LCFA-CoA, this relationship may be vital in determining the fate of LCFA-CoA between esterification and storage as TG or transport across the mitochondrial membrane and beta oxidation. However,

GPAT: glycerol-3-phosphate acyltransferase

DGAT: diacylglycerol acyltransferase

relatively little information is available regarding the regulation of the TG-synthesizing enzymes in skeletal muscle in health and disease and the impact of exercise and nutrition upon it.

At rest, 50% to 60% of FAs taken up by the muscle are esterified into IMTG (94), and the majority of FAs oxidized originate from plasma-derived FA. However, during one-legged knee extensor exercise, 85% of FAs taken up into muscle are oxidized, and the rate of FA esterification is much lower in the exercised leg (94). A similar decrease in FA esterification has been observed during electrical stimulation of isolated rat soleus muscle (27). It has been suggested that such reductions in TG synthesis in skeletal muscle during exercise can be attributed to elevations in AMPK activity (36). Conversely, elevations in both DAG and TG synthesis in soleus muscle in vitro occur in response to insulin (27). This suggests that the activity of both the proximal (GPAT) and distal (DGAT) enzymes of IMTG synthesis is increased in response to feeding. Furthermore, the shuttling of FA toward esterification is reduced following treatment with PI3 kinase inhibitors (27, 80), and therefore insulin stimulation of IMTG synthesis appears to be mediated via the PI3 kinase pathway.

Increased content of TG-synthesizing enzymes such as mtGPAT and DGAT1 after a single bout of aerobic exercise (95) explains the supercompensation of IMTG concentrations that occurs following IMTG depletion during exercise and provided adequate fat is ingested in the postexercise period. Such an upregulation of IMTG synthesis after exercise also exerts a protective effect on skeletal muscle. The evidence suggests that incorporation of the lipid metabolites LCFA-CoA and DAG to inert TG increases insulin sensitivity. For example, feeding fat after exercise increases IMTG content but does not impair insulin sensitivity (32). Additionally, the upregulation of IMTG synthesis after an acute bout of exercise prevents any decrement in insulin action in response to an overnight lipid infusion, an intervention that usually induces insulin resistance (95). Similar protective effects upon insulin sensitivity have been shown after myocellular DGAT1 overexpression in rats (73) leading to increased IMTG, and reduced DAG and ceramide concentrations and maintenance of insulin sensitivity following a high-fat diet. Recently, similar effects of DGAT1 upregulation have also been observed in heart muscle (74).

The increase in IMTG synthesis (through either overexpression of TG-synthesizing enzymes or acute exercise) appears to explain the paradox that the muscles of athletes are insulin sensitive despite having elevated IMTG concentrations (37). To date, there is limited evidence from human studies regarding the TG synthetic ability in skeletal muscle in different populations. A very recent study showed no difference in mtGPAT or DGAT1 protein content between sedentary lean and obese individuals (118) despite the obese individuals having elevated concentrations of ceramide and insulin resistance. It was postulated that an increase in plasma FA uptake in obesity (9) is not matched by enhanced IMTG synthesis, and this inadequate handling of intramuscular FA subsequently leads to an increase in lipid metabolites (in this case ceramide).

INTRAMUSCULAR TRIACYLGLYCEROL AND CHRONIC EXERCISE

Exercise Training and IMTG Content

As mentioned above, there are apparent relationships between physical activity and IMTG concentrations on the one hand and between obesity and IMTG concentrations on the other hand. Both are mechanistically linked to insulin sensitivity but with opposing effects. Initial studies demonstrated a positive association between IMTG content and insulin resistance in sedentary lean and obese subjects (82, 86), whereas trained individuals combine a high IMTG content with muscles that are highly sensitive to insulin (37, 122).

Cross-sectional studies looking at the full spectrum of insulin sensitivity and aerobic

fitness show a U-shaped relationship with IMTG content, with high concentrations of IMTG present in both obese insulin-resistant individuals and trained highly insulin-sensitive athletes; the "normal" population has lower concentrations of IMTG and moderate insulin sensitivity (37, 78, 122). It is clear that the accumulation of IMTG is an adaptation that may fit multiple metabolic aims. In athletes, IMTG accumulation along with elevated oxidative capacity is a training adaptation to provide the optimal availability of lipid fuel during exercise. In obese sedentary individuals, IMTG accumulation is an adaptation to the excess delivery and uptake of fatty acids into the muscle (9), and it has been speculated that IMTG accumulation in these individuals is an attempt to protect the muscle from the accumulation of lipotoxic fatty acid metabolites that lead to insulin resistance (131).

Although the results from cross-sectional studies on training and IMTG content are clear, the results of longitudinal training studies are less clear. Studies have shown an increase (26, 62, 87, 89, 99, 117), a decrease (2, 4, 11, 12, 106), or no change (34, 47, 53) in IMTG content in response to regular exercise in both lean and obese insulin-resistant individuals. An attractive hypothesis is that regular exercise will stimulate IMTG synthesis, which will increase the incorporation of fatty acids into the IMTG pool, and the resultant reduction in LCFA-CoA, DAG, and ceramide concentrations will improve insulin sensitivity. Dube et al. (26) demonstrated results in favor of this hypothesis following 16 weeks of aerobic training in older overweight nondiabetics. However, other studies have failed to observe increases in IMTG content despite improvements in insulin sensitivity. It is possible that the balance between IMTG lipolysis and IMTG synthesis is reset, leading to lower LCFA-CoA, DAG, and ceramide concentrations at a range of IMTG concentrations. Furthermore, differences in subject characteristics, training protocols, diet, and method of IMTG analysis may also explain the apparent discrepancies among a number of studies. Diet composition in particular has a huge influence on IMTG content and may well determine the IMTG response to training. IMTG content is unlikely to increase during calorie or fat restriction that induces a negative energy balance, and a substantial intake of dietary fat may be required to observe increases in IMTG content with training.

The finding that well-trained endurance athletes have an elevated IMTG content is consistent, but it is not known if increases in the content or activity of the enzymes involved in TG synthesis are elevated in these individuals to explain such increases. No changes in DGAT1 protein were seen after an eight-week endurance training program in young healthy males (2). It is possible that the increase in IMTG content is fully explained by transient increases in the rate of TG synthesis in the post-exercise period. Today it is not known whether this is a result of allosteric activation, phosphorylation of activation sites, transient increases in gene expression and protein content of the TG-synthesizing enzymes, or increased fatty acid supply to the trained muscle as a result of higher lipoprotein lipase activities in the capillary bed of skeletal muscle.

IMTG Utilization During Exercise After Training

The primary metabolic adaptation to endurance training is an increased oxidation of fatty acids and a reduced reliance on carbohydrate stores. This shift in fuel utilization is associated with an increase in mitochondrial density and content of oxidative enzymes in addition to increases in sarcolemmal and mitochondrial FA transport capacities. Circumstantial evidence suggests that the ability to oxidize IMTG stores during exercise is also improved after training, as depletions during exercise are more consistently shown in trained individuals with a variety of techniques. Furthermore, IMTG oxidation during exercise is only minimal in sedentary individuals (98), and there is no decrease in IMTG concentrations following exercise in obese type 2 diabetics (124).

Longitudinal studies have been employed to investigate training-induced changes in IMTG metabolism. Schrauwen et al. (98) demonstrated that after three months of low-intensity exercise training in formerly sedentary individuals, the increase in fat oxidation observed during exercise was almost fully attributable to increases in the oxidation of TG-derived fatty acids. Similar results have been demonstrated by other studies also using tracer methodology to estimate TG oxidation during exercise after training (53, 63, 75, 87); however, it is not clear from these studies whether this increase in TG oxidation is due to an increased oxidation of IMTG or lipoprotein-TG, or a mixture of the two. Studies measuring IMTG with biochemical methods do not show consistent increases in IMTG use after exercise training (4, 62, 109). It is again possible that the method of IMTG analysis as well as subject variation, training protocols, and gender differences may explain discrepancies between studies.

Intramuscular lipid droplets are preferentially located in close vicinity of the mitochondria, with the lipid droplets filling some of the spaces in the mitochondrial network. This allows for the efficient transport and oxidation of fatty acids liberated by lipolysis of IMTG during exercise (50, 102). The percentage of LDs that are located in contact with the mitochondria increases after a period of training (117), and spatial arrangement of lipid droplets in the direct vicinity of mitochondria may partly explain the increase in IMTG oxidation with training.

It has been reported that trained individuals exhibit greater rates of skeletal muscle lipolysis (shown by higher rates of glycerol release from skeletal muscle) during exercise in comparison to untrained individuals (19). The differences in muscle lipolytic rate during exercise do not appear to be explained by changes in the content or activity of HSL. A period of endurance training in rats (30) and humans (2) has no effect upon HSL content. Furthermore, another study did not observe differences in lipase activity during exercise between trained and untrained individuals despite a tendency for a higher IMTG recruitment in the trained individuals (46). Although there does not appear to be a training-induced increase in HSL protein content, evidence indicates that ATGL content increases following eight weeks of endurance training without a change in the content of the ATGL coactivator, CGI-58 (2).

There is some evidence that impairments exist in the lipolytic response of skeletal muscle in obese insulin-resistant individuals. One study demonstrated a lower HSL content and reduced adrenergic-mediated muscle lipolysis in the forearm of obese insulin-resistant individuals (6). This potentially explains why IMTGs are not utilized during exercise in type 2 diabetic individuals (124). It is possible that the elevated concentrations of plasma FA and of intramuscular LCFA-CoA limits lipolysis in type 2 diabetes (124, 133). Furthermore, there is also evidence that an imbalance exists between TG and DAG hydrolysis in obesity. This could be very important in the development of insulin resistance in sedentary obese individuals. A greater ratio of TG/DAG hydrolase activity was observed in obesity, and such an imbalance led to the accumulation of DAG and ceramide in the skeletal muscle (79). The incomplete degradation of IMTG could explain why lipid metabolites accumulate in the muscle, resulting in insulin resistance.

DIET, INTRAMUSCULAR TRIACYLGLYCEROL, AND LIPID METABOLITES

High-Fat Feeding, IMTG, and Insulin Sensitivity

Many studies have demonstrated that significant increases in IMTG content can be achieved through a period of high-fat feeding. High-fat diets providing 50% to 60% of energy from fat for a period of one to five weeks increases IMTG content by 50% to 100% (101, 129). As expected, such increases in IMTG are greater in oxidative muscle fibers (3). Furthermore, combining endurance exercise training with the consumption of a high-fat diet

does not prevent the increase in IMTG content (48). The increase in IMTG content appears to be an early adaptive response to high-fat feeding (>60% of the energy derived from fat), as smaller increases in IMTG content of ~40% to 50% are already achieved within 24 to 60 hours after the start of high-fat feeding (3, 57, 108, 112, 143). Therefore, the IMTG pool in lean individuals appears to be a flexible energy store that can be expanded during periods of lipid oversupply.

Dietary fat appears to be necessary for the replenishment of IMTG stores after endurance exercise leading to IMTG depletion. A normal diet (~35% energy as fat) ingested in the period after endurance exercise appears to contain enough lipid to fully replenish IMTG stores within 22 to 48 hours (70, 125). In a manner similar to muscle glycogen repletion, IMTG concentrations have been shown to be supercompensated in the postexercise period with the ingestion of either moderate-fat (35%) (70) or high-fat diets (55%) (25). Conversely, if a carbohydrate-rich diet is consumed after exercise and fat intake is restricted (10% to 24% of energy from fat), the repletion of IMTG is substantially impaired and IMTG content remains below pre-exercise levels until 72 hours after exercise (25, 70, 125). This could have fuel selection implications for athletes who consume a high-carbohydrate/low-fat diet in an attempt to maximally replenish muscle glycogen stores after exercise, and may result in prolonged long-term depletion of IMTG. Although the deleterious effects of low muscle glycogen on endurance exercise performance are well described (5), the performance effect of exercising in an IMTG-depleted state is not known today. Interestingly, replenishment of IMTG content following resistance exercise appears to be rapid and occurs within two hours of exercise without additional fat intake (64), suggesting that adipose tissue lipolysis after resistance exercise (16) is responsible for the redistribution of the endogenous fat stores.

The increase in lipid availability from a period of high-fat feeding induces an increase in IMTG content. Similar increases in IMTG content can also be elicited through a period of starvation (57, 107). For example, a study by Johnson et al. (57) investigated the effect of 67 hours of either high-fat feeding or starvation. Both protocols elicited similar increases in IMTG compared to a normal mixed diet. The related increases in adipose tissue lipolysis, plasma FA, and plasma TG in response to both starvation and high-fat feeding appear to mediate the increases in IMTG content. It has been demonstrated a number of times that increasing plasma FA concentrations through intralipid and heparin infusion results in the accumulation of IMTG and other intramuscular lipid species (DAG and ceramide) (14, 142). Similarly, the elevations in plasma FA during prolonged endurance exercise also coincide with increases in the IMTG content in nonexercising muscles (100).

Changes in substrate metabolism and increases in fat oxidation occur within hours of high-fat feeding (35, 84). In addition, several studies have demonstrated that high-fat diets, which elevate IMTG concentrations, also impair insulin sensitivity. For example, two to three days of high-fat feeding in sedentary individuals has been shown to reduce insulin sensitivity (3, 112) and glucose tolerance (83). Furthermore, periods of starvation are also linked to reductions in insulin sensitivity (57). The exact cause of the reductions in insulin sensitivity in response to high-fat feeding (and starvation) has not been determined, but it is unlikely to be a result of elevations in IMTG content per se. Many studies have investigated the mechanisms of fat-induced insulin resistance by raising plasma FA concentrations through infusion of intralipid and heparin and have observed accumulations of DAG and ceramide, reductions in insulin-mediated glucose uptake and impairments in insulin signaling. However, whether similar mechanisms contribute to the impairments in insulin action after acute high-fat feeding remains to be determined. Another well-known textbook mechanism by which an increase in plasma FA and lipid availability has been proposed to reduce glycolysis and glucose oxidation is the Randle

cycle (91). The possibility that these mechanisms operate in parallel cannot be excluded.

Dietary Fatty Acid Composition, IMTG, and Insulin Sensitivity

It also appears that the composition of dietary fats can affect insulin sensitivity; however, to date the majority of these studies have been carried out in vitro. Several studies have demonstrated that muscle cells in vitro become insulin resistant when incubated in saturated fatty acids, and in long-chain saturated fatty acids in particular (17, 97). In these studies, the inhibition of insulin action and insulin signaling associated with saturated FA incubation was related to increases in DAG and ceramide concentrations. In contrast, incubation with unsaturated fatty acids (oleate, linoleate) failed to induce insulin resistance and had no effect on DAG and ceramide concentrations (17, 97). Similarly, rats fed a high-saturated-FA diet also exhibited elevated muscle DAG concentrations and developed insulin resistance in comparison to rats fed polyunsaturated fatty acid (71). Furthermore, there is some evidence in vitro and from animal work in vivo that unsaturated fatty acids stimulate IMTG synthesis to a greater extent than do saturated fatty acids, which may explain the lower DAG concentrations and maintenance of insulin sensitivity when elevating the availability of unsaturated fatty acids (21, 71). In addition, the induction of insulin resistance in skeletal muscle through the ingestion of saturated fatty acids also appears to be linked to activation of (pro)inflammatory pathways in skeletal muscle (discussed in 59, 127). Frangioudakis et al. (33) did not observe impairments in insulin-mediated signaling in skeletal muscle in response to chronic feeding of both saturated and n-6 polyunsaturated fatty acids in rats.

However, a large-scale study in which 162 human participants followed a diet high in either saturated fatty acids or monounsaturated fatty acids for three months found that insulin sensitivity was 12.4% lower on the diet high in saturated fatty acids and 8.8% higher on the diet high in monounsaturated fatty acids (128). Interestingly, each of these diets consisted of 37% fat, which is typical of a western diet, but increasing the saturated fatty acid composition still reduced insulin sensitivity, indicating that the composition of fatty acids (whether saturated or unsaturated) is a major factor in determining the development of insulin resistance.

Thus it would seem that although high-fat diets lead to IMTG accumulation, they do not by definition lead to increases in intramuscular lipid metabolites and insulin resistance. It would appear that diets high in saturated fat do lead to DAG and ceramide accumulation and, along with activation of inflammatory signaling pathways, subsequently impair insulin signaling. In contrast, unsaturated fatty acids have been reported to increase insulin sensitivity at the whole-body level in humans, but further work is required to confirm whether the mechanism behind this effect involves increased IMTG synthesis and lowering of lipid metabolites in human muscle.

Calorie Restriction and Insulin Sensitivity

There is substantial evidence to suggest that calorie restriction interventions can decrease IMTG and also improve insulin sensitivity. IMTG concentration decreased in patients with type 2 diabetes following 16 weeks of chronic calorie restriction (800–1200 kcal/day), resulting in ~15 kg weight loss (38). Similarly, another robust calorie restriction (700 kcal/day) protocol for only six days led to pronounced reductions in IMTG (40% to 56%) and small decreases in body mass index in both type 2 diabetics and obese nondiabetics (69). In addition, glucose disposal rate was increased by 9.3% (69). A recent study showed a nonsignificant 23% reduction in IMTG concentrations after 53 days of a very-low-calorie diet, inducing ~10 kg reduction in body weight (90). Furthermore, the reversal of insulin resistance in obese subjects after bariatric surgery (enforced calorie restriction) is associated with a reduction in IMTG (40). These studies

demonstrate that IMTG content can be reduced relatively quickly when energy intake is severely restricted. Reductions in IMTG content have not always been observed with more moderate calorie-restriction protocols. Two studies employing 1200–1600 kcal/day interventions in type 2 diabetics failed to observe reductions in IMTG concentrations (85, 116). It is possible that reductions in IMTG content occur only upon severe calorie restriction, because the methods used to investigate changes in IMTG concentrations are unable to detect the smaller reductions in IMTG in response to less severe calorie restriction. Studies that have used a combination of calorie restriction and exercise training have also demonstrated reductions in IMTG. A reduction in IMTG (19%) was found in the tibialis anterior using nuclear magnetic resonance, but only when calorie restriction was combined with exercise, which was also successful in improving insulin sensitivity by 57% (116). Furthermore, Solomon et al. (106) combined 12 weeks of aerobic exercise training with a similar calorie restriction (~1300 kcal/day) and observed a reduction in IMTG concentrations as well as improvements in insulin sensitivity.

Other groups have looked more specifically at the changes seen in IMTG morphology (45). Four months of weight loss via caloric restriction (decrease by 500–1000 kcal/d) and exercise intervention resulted in a ~10% weight loss. Interestingly, in this study, total IMTG content was unchanged, but lipid droplet size was decreased following the exercise and weight-loss regime. This decrease in lipid droplet size was correlated with an increase in insulin sensitivity (45). Taken together, these results show that there is a marked reduction in IMTG content in response to extreme calorie restriction, and it is likely that a more subtle reduction in IMTG content with more conservative calorie-restriction protocols are sometimes not detected using certain methods of IMTG analysis. These interventions often improve insulin action, although the reduction in IMTG per se is unlikely to explain such improvements. Rather, the reduction in IMTG content may coincide with reductions in other lipid species (DAG and ceramide) and reductions in lipid droplet size, which both appear to be important for improved insulin sensitivity.

CONCLUSIONS

IMTGs are a dynamic fatty acid storage depot that can be utilized during periods of elevated energy demand and can consume excess plasma and intramuscular fatty acids during periods of elevated lipid availability (see **Figure 3**). There is an accumulating amount of evidence that the metabolic adaptations to endurance exercise that result in improved IMTG metabolism exert a protective effect upon muscle insulin sensitivity. Trained individuals have a higher capacity for fat oxidation, the majority of which is derived from elevated IMTG oxidation. Therefore, those involved in regular exercise regularly deplete IMTG stores and also regularly have high uptake and oxidation rates of plasma-derived fatty acids. Alternatively, in the period after exercise, IMTG will remain low until dietary fat is ingested, at which point IMTG synthesis rates will be elevated. Therefore, during periods of elevated lipid availability that are associated with high plasma FA uptake into skeletal muscle and the development of insulin resistance in sedentary individuals, the FA will be efficiently channeled into IMTG in trained individuals. This regular turnover of IMTG with regular depletion and replenishment cycles will maintain a mobile IMTG pool with small, metabolically flexible lipid droplets, which will aid the maintenance of low concentrations of LCFA-CoA, DAG, and ceramide and a high insulin sensitivity in skeletal muscle.

Alternatively, sedentary obese individuals rarely experience exercise-induced increases in FA oxidation and appear unable to utilize IMTG during exercise. Furthermore, fatty acid incorporation into IMTG will be reduced during elevated lipid availability due to the lack of both regular IMTG depletion and exercise-induced stimulation of IMTG synthesis. This will result in the development of a static IMTG pool with enlarged and inflexible lipid droplets

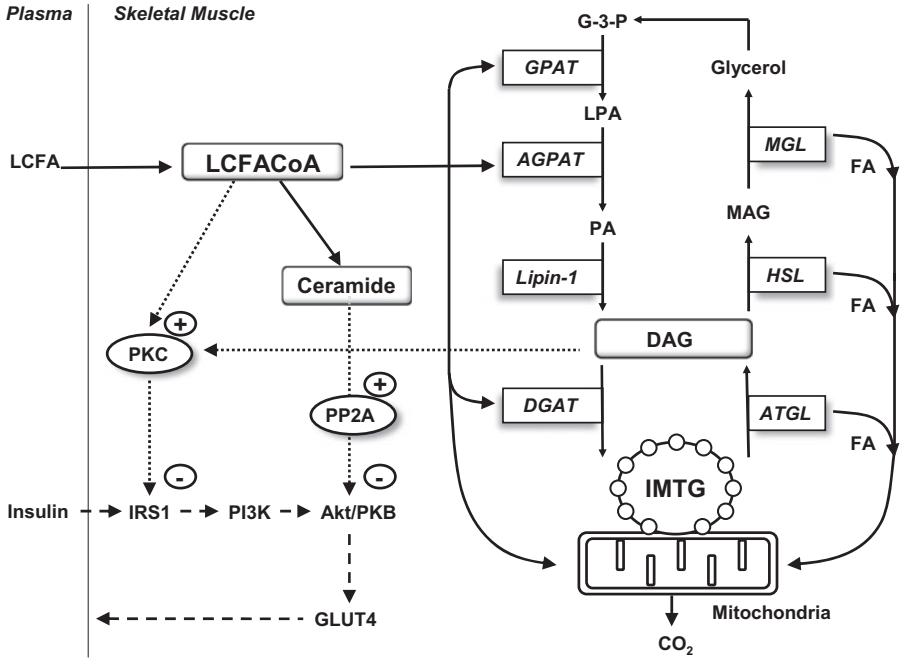

Figure 3

Schematic representation of how intramuscular triacylglycerol (IMTG) metabolism in skeletal muscle determines the concentrations of lipid metabolites and insulin sensitivity. Long-chain fatty acids (LCFAs) are transported into the muscle and activated to long-chain fatty acyl-CoA (LCFA-CoA), which can enter IMTG synthesis by their sequential addition onto a glycerol-3-phosphate backbone. IMTG lipolysis generates diacylglycerol (DAG) and fatty acids (FAs), which can be oxidized by the mitochondria, re-esterified, or mediate insulin sensitivity. LCFA-CoA and DAG activate PKC, which stimulates serine phosphorylation and inactivation of insulin receptor substrate 1 (IRS1), whereas ceramide activates protein phosphatase 2 (PP2A), which dephosphorylates and inactivates Akt/protein kinase B (Akt/PKB). Downregulation of the insulin-signaling pathway reduces the translocation of glucose transporter 4 (GLUT4) to the plasma membrane and inhibits insulin-stimulated glucose transport. AGPAT, acylglycerol phosphate acyltransferase; ATGL, adipose triglyceride lipase; DGAT1, diacylglycerol acyltransferase 1; G-3-P, glycerol-3-phosphate; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; LPA, lysophosphatidic acid; MAG, monoacylglycerol; MGL, monoacylglycerol lipase; PA, phosphatidic acid; PKC, protein kinase C; PI3K, phosphoinositide 3-kinase.

that do not undergo regular depletion and replenishment cycles. Plasma fatty acid delivery and uptake into skeletal muscle is elevated in obesity due to the enlarged adipose tissue mass and, due to the inefficient oxidation and/or storage of the incoming fatty acid, results in the accumulation of lipid metabolites in skeletal muscle. Accumulations of intramuscular lipid metabolites will exacerbate impairments in IMTG metabolism due to a downregulation of HSL activity and reduced clearance of DAG. Furthermore, impairments in insulin signaling may also inhibit insulin stimulation of IMTG synthesis in addition to the well-described impairments in insulin signaling, GLUT4 translocation, and glucose uptake. Resultant hyperglycemia and hyperinsulinemia are likely to lead to the progressive decline of glycemic control. Therefore, the combination of a sedentary lifestyle and consumption of diets high in fat, and saturated fats in particular, not only leads to the development of obesity but also to the development of metabolic impairments in skeletal muscle that contribute to whole-body disease states such as type 2 diabetes and cardiovascular disease.

SUMMARY POINTS

1. In lean active individuals, the IMTG pool is regularly depleted during exercise and is replenished during subsequent feeding. Dietary fat is required to fully replenish IMTG stores, which can be supercompensated after exercise.
2. Reduced IMTG oxidation appears to contribute to the reduction in fat oxidation during exercise in sedentary individuals. The low turnover of the IMTG pool likely contributes to the accumulation of lipid metabolites and insulin resistance in skeletal muscle.
3. Muscle lipolysis is increased during exercise by activation of HSL and ATGL by mechanisms that are only partially known today and that may also involve coactivators and the LD-associated proteins.
4. A high IMTG synthesis rate is a major determinant of IMTG content and keeps lipid metabolite concentrations low and insulin sensitivity high.
5. IMTG synthesis rates can be increased by exercise, dietary interventions, and combined dietary and exercise interventions. Recent studies suggest that fatty acid composition of the diet is more important than total fat intake in the development of skeletal muscle insulin resistance.

FUTURE ISSUES

1. How are the enzymes of IMTG synthesis regulated and spatially distributed? Are there differences in the regulation and/or spatial distribution of these enzymes between lean active individuals and sedentary obese individuals?
2. How are the enzymes of skeletal muscle lipolysis regulated at rest and during exercise? Are other lipolytic enzymes involved? What is the role of LD-associated proteins and other activators or inhibitors?
3. How do muscle lipolysis and IMTG synthesis interact and lead to a rise in the concentration of lipid metabolites and the development of insulin resistance?
4. What are the optimal exercise and nutritional interventions and pharmacological target enzymes to enhance IMTG turnover and alleviate insulin resistance in previously sedentary individuals?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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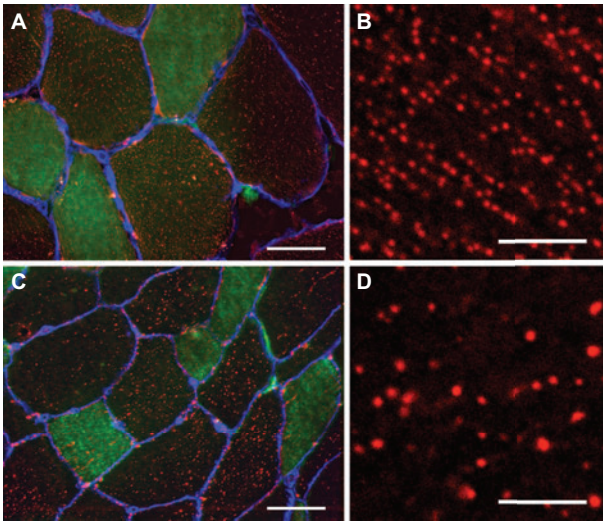

Figure 1

(*A*, *B*) Immunofluorescence staining of IMTG in human muscle biopsies taken from a young male trained cyclist (age, 28 years; BMI, 23.6 kg/m^2) and (*C*, *D*) an older sedentary male (age, 53 years; BMI, 28.9 kg/m^2) using the method described by Shaw et al. (102). Widefield images (*A* and *C*; bar = 50 μm) show a greater intramuscular triacylglycerol (*red*) content in type 1 muscle fibers (fibers positively stained green for myosin heavy chain type 1). Higher magnification confocal images of oil red O staining (*B* and *D*; bar = 10 μm) show a large number of small lipid droplets in the trained cyclist. The older sedentary male shows fewer lipid droplets, but droplets are of a larger size (C.S. Shaw, J. Clark, & A.J. Wagenmakers, unpublished observations). BMI, body mass index.

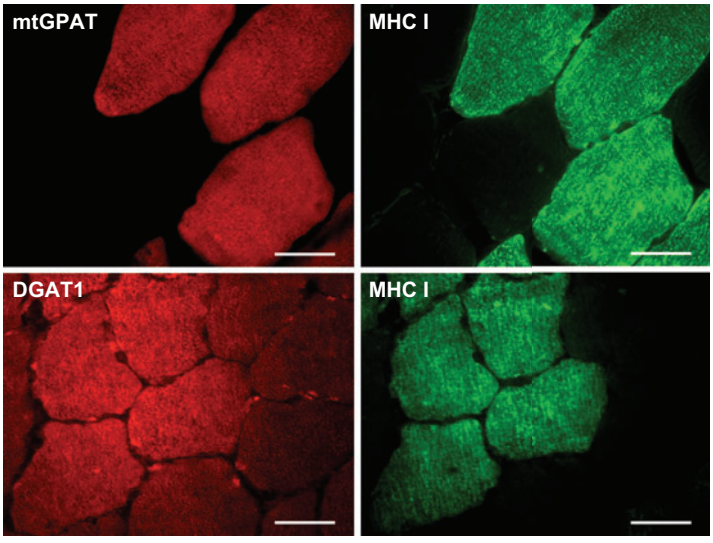

Figure 2

Immunofluorescence staining of mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) and diacylglycerol acyltransferase (DGAT1) in combination with myosin heavy-chain type 1 (MHC I) to detect type 1 muscle fibers in a human muscle biopsy. The top panel shows mtGPAT to be primarily present in type 1 muscle fibers (*green*). The lower panel shows that DGAT1 is present in both type 1 (*green*) and type 2 (*unstained*) muscle fibers, with a greater DGAT1 signal in type 1 fibers (J. Clark, C.S. Shaw, & A.J.M. Wagenmakers, unpublished observations).

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