Determinants of intramyocellular triglyceride turnover: implications for insulin sensitivity

Cédric Moro, Sudip Bajpeyi, and Steven R. Smith

Pennington Biomedical Research Center, Molecular and Experimental Endocrinology Laboratory, Baton Rouge, Louisiana

Moro C, Bajpeyi S, Smith SR. Determinants of intramyocellular triglyceride turnover: implications for insulin sensitivity. Am J Physiol Endocrinol Metab 294: E203–E213, 2008. First published November 14, 2007; doi:10.1152/ajpendo.00624.2007.—Increased intramyocellular triglyceride (IMTG) content is found in both insulin-sensitive endurance-trained subjects and insulin-resistant obese/type 2 diabetic subjects. A high turnover rate of the IMTG pool in athletes is proposed to reduce accumulation of lipotoxic intermediates interfering with insulin signaling. IMTG turnover is a composite measure of the dynamic balance between lipolysis and lipid synthesis; both are influenced by mitochondrial fat oxidation and plasma free fatty acid availability. Therefore, more attention should be given to the factors controlling the rate of turnover of IMTG. In this review, particular attention has been given to muscle oxidative capacity, plasma free fatty acid availability, and IMTG hydrolysis (lipolysis) and synthesis. A higher oxidative, lipolytic, and lipid storage capacity in the muscle of endurance-trained subjects reflects a higher fractional turnover of the IMTG pool. Thus the co-localization of intramyofibrillar lipid droplets and mitochondria allows for a fine coupling of lipolysis of the IMTG pool to mitochondrial β-oxidation. Conversely, reduced oxidative capacity and a mismatch between IMTG lipolysis and β-oxidation might be detrimental to insulin sensitivity by generating several lipotoxic intermediates in sedentary populations including obese/type 2 diabetic subjects. Further studies are clearly required to better understand the relationship between the rate of turnover of IMTG and the accumulation of lipotoxic intermediates in the pathophysiology of insulin resistance.

lipolysis; exercise; lipid storage; type 2 diabetes; skeletal muscle

SKELETAL MUSCLE LIPIDS have been considered as a potential substrate source for energy needs since the end of the 1950s (80). In studies on isolated rat diaphragm incubated ex vivo without glucose or fatty acid substrate, Neptune et al. (80) noticed that the respiratory quotient was quite low, ranging from 0.72 to 0.76, suggesting both a reliance on lipid as substrate and utilization of intracellular lipid stores as a fuel source. It is now largely accepted that intramyocellular triacylglycerol (IMTG) constitutes a significant source of energy in the body that can be used at rest and under circumstances of increased energy expenditure such as exercise (extensively reviewed in Refs. 60, 101, 123, 124, 131).

A clear relationship between IMTG accumulation and insulin resistance has been reported in obesity and type 2 diabetes (56, 60, 89, 97). However, IMTG content is also increased in endurance-trained subjects with high insulin sensitivity, suggesting that IMTG per se does not cause muscle insulin resistance (35). This difference may be directly linked to a higher turnover of IMTG in active endurance-trained individuals, i.e., increased depletion/repletion cycles of the IMTG pool and coupling of lipolysis to lipid oxidation. IMTG turnover is a composite measure of the dynamic balance between lipolysis and lipid synthesis; both are highly dependent on mitochondrial fat oxidation (ATP demand) and plasma free fatty acid availability. More specifically, fatty acids esterified in the IMTG pool are hydrolyzed by triglyceride lipases before oxidation, while they can be recycled back into the IMTG pool when not oxidized. Factors controlling the rate of turnover of IMTG include 1) muscle oxidative capacity, 2) IMTG breakdown (lipolysis), 3) free fatty acid (FFA) availability, and 4) (re)esterification of intermediate products such as diacylglycerol (DAG). Each of these is potentially important because of the hypothesized links between lipid intermediates and insulin sensitivity. In the present review, the term insulin sensitivity refers to maximal insulin-stimulated glucose disposal measured by hyperinsulinemic euglycemic clamp in vivo and 2-deoxyglucose uptake in vitro.

The purpose of this review is 1) to critically examine the present knowledge regarding the machinery needed for IMTG breakdown and synthesis, 2) to highlight advances in our understanding of the systems that regulate IMTG turnover, and 3) to identify gaps in our knowledge base to guide future research.

IMTG, OXIDATIVE CAPACITY, AND INSULIN RESISTANCE

Relationship between IMTG and insulin resistance in humans. Insulin sensitivity has been shown to be inversely related to IMTG content in sedentary populations (12, 51, 52, 68, 83, 87) (Fig. 1). Several molecular mechanisms have been suggested to explain this relationship. First, a higher level of IMTG deriv-
Ceramide in C2C12 muscle cells causes insulin resistance (107). An increased level of ceramide has been reported with obesity (2), while 8 wk of endurance exercise training decreased DAG and ceramide content in an obese population and increased insulin sensitivity but did not alter IMTG content (17). This is consistent with recent studies in rat showing a reduction in DAG and ceramide content in the muscle after 4–8 wk of exercise training with muscle triglyceride content not affected (73, 114). These data suggest that the higher IMTG content in a sedentary insulin-resistant population is probably not a direct cause of insulin resistance; rather, lipid intermediates are probably more important.

**Relationship between oxidative capacity and insulin sensitivity.** Skeletal muscle oxidative capacity, on the other hand, has been shown to be predictive of insulin action when sedentary and physically active individuals are studied (16). A reduction in fatty acid oxidation in skeletal muscle has been reported in obese and type 2 diabetic individuals in postabsorptive conditions (9, 10, 65, 112). This observation is more controversial when subjects are performing exercise where whole body fat oxidation has been found to be either reduced (86) or increased (14, 15, 37) compared with lean control. This reduced fat oxidation has been linked with the accumulation of IMTG and other lipid metabolites, which are then hypothesized to cause insulin resistance (35, 49, 68, 83). Several studies have shown that obesity and type 2 diabetes are associated with a lower percentage of oxidative (type I) and a higher percentage of glycolytic (type Iib) muscle fibers (47, 81, 119). Moreover, type I muscle fibers are more insulin sensitive compared with type Iib muscle fibers (46, 47, 59, 74). Endurance-trained athletes, on the other hand, have higher mitochondrial content and oxidative capacity (21, 35, 53, 95) and are capable of oxidizing lipids to a greater extent compared with a sedentary population (54) (Fig. 1). Several studies indicate that IMTG is an important source of energy for skeletal muscle during exercise (67, 69, 109). Highly trained male endurance runners use more fat as fuel during low-intensity exercise than do untrained healthy men. This increase in fat oxidation is thought to be partly derived from IMTG stores (50, 67). IMTG was also shown to have an important contribution in providing fuel for muscle metabolism in the postexercise recovery period (63). Decombe et al. (27) reported a 71% higher concentration of IMTG in trained compared with untrained individuals. The overall increase in lipid oxidation with exercise is paralleled by an increased number of mitochondria and mitochondrial enzymes with training (17). In summary, the inverse relationship between IMTG and insulin sensitivity is not evident when endurance-trained subjects are considered. This suggests that IMTG is a marker of insulin resistance; the true culprit is most likely the increased intramyocellular availability of toxic lipid intermediates.

**Mitochondrial dysfunction and insulin resistance.** Obesity and type 2 diabetes have been associated with reduced mitochondrial content and/or defects in mitochondrial function in skeletal muscle, which in turn may be responsible for the reduced rate of mitochondrial oxidative phosphorylation and IMTG turnover, leading to decreased oxidation of IMTG and plasma FFA and increasing the availability of toxic lipid intermediates (Fig. 1). Electron microscopy shows that intermyofibrillar and subsarcolemmal mitochondria are smaller and less dense, and that electron transport chain (ETC) activity is

---

**Fig. 1.** Model for the relationship between intramyocellular triglyceride (IMTG) content, oxidative capacity, mitochondrial content, and insulin sensitivity in humans. In this model, it is shown how environmental factors can modify this relationship by means of changes in lifestyle and diet and/or therapeutic interventions. IMCL, intramyocellular lipid; T2DM, type 2 diabetes.  

**Notes:**
- **Exercise training** and **Calorie restriction** have been shown to increase oxidative capacity and mitochondrial content, as well as improve insulin sensitivity.  
- **Insulin-sensitizing drugs** have been shown to decrease IMTG content and improve insulin sensitivity.  
- **Inactivity** and **Obesity** have been shown to decrease oxidative capacity and mitochondrial content, as well as decrease insulin sensitivity.  
- **High fat diets** have been shown to increase IMTG content and decrease oxidative capacity and mitochondrial content, as well as decrease insulin sensitivity.  
- **Athlete** has been shown to increase oxidative capacity and mitochondrial content, as well as increase insulin sensitivity.  

**Conclusion:** The model suggests that changes in lifestyle and diet and/or therapeutic interventions can modify the relationship between IMTG content, oxidative capacity, mitochondrial content, and insulin sensitivity.
Reduced in the skeletal muscle of obese and type 2 diabetic individuals (57, 96). Thus resting mitochondrial ATP production was found to be reduced by ~30% in the muscle of insulin-resistant subjects when compared with insulin-sensitive matched control subjects (88).

Another approach to explore the link between mitochondrial function and insulin resistance is to intervene, in the case of insulin-resistant subjects, with exercise training, weight loss, or a combination of both. Menshikova et al. (77) studied the relationship between skeletal muscle mitochondrial ETC activity and insulin sensitivity before and after a 16-wk combined weight loss and exercise intervention in middle-aged overweight and obese volunteers. ETC activity (adjusted to creatine kinase content) increased by 2-fold and 1.5-fold in the subsarcolemmal (SSM) and intermyofibrillar (IMF) fractions, respectively, independently of changes in mitochondrial mass (mtDNA). This suggests an improvement in mitochondrial function rather than the proliferation of mitochondria in this particular setting. Interestingly, the increase in SSM ETC activity after intervention correlated with insulin sensitivity even after adjustment for aerobic capacity (VO2max) (77). In another study, Tarnopolsky et al. (120) reported that 7 wk of aerobic training in young lean men and women increased the number of lipid droplets and changed their intracellular distribution. Transmission electron micrograph revealed a reorganization of lipid droplets along the myofibrillar apparatus as well as an increase in their physical contact with mitochondria (120). Those functional changes in IMTG distribution were associated with an increase in total muscle area and in the capacity for fat oxidation during exercise and insulin sensitivity. Similarly, Toledo et al. (121) found a substantial increase in mitochondrial cardiolipin content (55%), a marker of mitochondrial inner membrane surface area, together with an improvement in insulin sensitivity (59%) in type 2 diabetic subjects after an intervention of daily moderate-intensity exercise combined with moderate weight loss. However, the intervention design does not allow us to distinguish whether the mitochondrial dysfunction is a primary cause in insulin resistance or a secondary physiological adaptation to reduced physical activity. Further studies are required to specifically address this issue.

**REGULATION OF IMTG BREAKDOWN**

**IMTG and lipid droplet-associated proteins.** Lipid droplet-associated (LD) proteins of the perilipin, adipophilin/adipose differentiation related protein (ADRP), and tail interacting protein of 47 kDa (TIP47) family (PAT family) sequester and regulate intracellular neutral lipid stores in most mammalian cells. PAT proteins have been most extensively studied in adipocytes where they play a key role in neutral lipid storage and lipolysis (115, 116, 137). In skeletal muscle, less is known. Prats et al. (94) demonstrated the presence of at least two proteins, ADRP (or adipophilin) and TIP47; both are associated with intermyofibrillar lipid droplets in rat skeletal muscle (Fig. 2). Perilipin is not detected at the mRNA level or at the protein level in human skeletal muscle (99, 137). The PAT family also includes other recently discovered members. S3-12 (138) was described in murine 3T3-L1 adipocytes and is expressed in mouse skeletal muscle. OXPAT, also called PAT-1 (137) or lipid storage droplet protein-5 (LSDP5) (26) or myocardial lipid droplet protein (MLDP) (139), was first characterized in highly oxidative tissues such as liver, heart, and skeletal muscle.

Ex vivo studies using various nonmuscle cell lines have shown that overexpression of PAT proteins inhibits lipolysis by preventing the access of lipases to the lipid droplet, contributing to accumulation of triacylglycerol (TAG) within the cell (115, 116, 137). Few studies have explored the relationship between PAT proteins and IMTG. In human skeletal muscle, Phillips et al. (91) observed a positive relationship between ADRP protein expression and insulin sensitivity in obese diabetic subjects before and after a combined weight loss and pharmacological intervention program. Overexpression of perilipin-A in human primary myoblasts leads to the accumulation of TAG without changing DAG or ceramide content (92). These studies suggest the hypothesis that increasing LD protein content in muscle prevents access of TAG lipases to the IMTG pool. This might reduce the production of lipotoxic intermediates (fatty acyl-CoA, DAG, and ceramides), thus improving insulin signaling within the muscle cell. In conclusion, little is known on the role of LD proteins in the regulation of IMTG turnover. Further studies are clearly required to identify the
muscle fiber type distribution and regulation of PAT proteins and their relationship to insulin sensitivity.

**Hormone-sensitive lipase: a major lipolytic enzyme in skeletal muscle.** The enzymatic regulation of the TAG pool in skeletal muscle was poorly understood before the identification of hormone-sensitive lipase (HSL). HSL is a neutral lipase of 84 kDa displaying an optimum activity at pH 7.0 and a 10-fold higher specific activity for DAG compared with TAG, monacylglycerol, and cholesterol ester (7). Pioneering studies in rat muscle found that HSL was expressed up to fourfold more at the protein level in oxidative muscles (soleus and diaphragm) compared with glycolytic muscles (extensor digitorum longus and epitrochlearis) (71). DAG hydrolase activity measured in the crude supernatant of isolated muscle fibers was eightfold higher than TAG hydrolase activity regardless of the type of muscle, whereas DAG and TAG hydrolase activity was threefold higher in oxidative muscles. Incubation of soleus muscle with a supraphysiological dose of epinephrine increased TAG hydrolase activity by 30%. Neutralization of HSL by an anti-HSL chicken serum only partly abolished both basal and epinephrine-stimulated TAG lipase activity. The residual activity may be due to another lipase(s). Neutral TAL lipase activity is also induced when rat soleus muscles are electrically stimulated for 1 h (70). More recently, Prats et al. (94) have shown by confocal imaging of single muscle fibers that HSL translocates to IMTG in response to epinephrine or electrically mediated contraction (Fig. 2). A similar mechanism occurs in adipose tissue during natriuretic peptide- and catecholamine-stimulated lipolysis in which HSL translocates to the lipid droplet and perilipin-A on phosphorylation by PKA (cAMP-dependent protein kinase) (19, 117) and PKG (cGMP-dependent protein kinase) (110). However, because of the absence of perilipin-A in human skeletal muscle, it is still unclear how HSL translocates to lipid droplets and which docking proteins are responsible for this molecular event. This demonstrates a key role for HSL in the contraction- or epinephrine-stimulated mobilization of IMTG stores, providing fuel during exercise, stress, or reduced energy supply (Fig. 2). Consistent with this view, HSL content is greater in muscles containing a higher proportion of type I oxidative fibers and IMTG (125).

**Activation of muscle HSL by contraction and epinephrine.** In vivo studies in bilaterally adrenalectomized subjects have shown that epinephrine is required for a full stimulation of HSL activity in muscle during exercise (66). HSL activity does not change during exercise in adrenalectomized patients, which suggests an important role of epinephrine in the regulation of HSL activation in skeletal muscle during exercise. In healthy recreationally active subjects, muscle HSL activity is dependent on both exercise intensity and duration. HSL activity is rapidly increased after 1 min of exercise at 30 and 60% VO₂peak and to a greater extent at 90% VO₂peak. HSL activity remains elevated after 10 min of exercise at 30 and 60% VO₂peak, while plasma epinephrine levels are unchanged, suggesting epinephrine-independent mechanisms in the recruitment of HSL to lipid droplet (130). Although the precise mechanisms are unknown, this suggests that contraction-related factors, potentially Ca²⁺ signaling, phosphorylate and activate HSL early during exercise. More recently, it has been proposed that extracellular-related kinase-1/2 (ERK1/2) phosphorylation is rapidly increased during exercise and may mediate the contraction-induced increase in HSL activity early during exercise (136). Additional experiments demonstrated that contraction-induced HSL activity is abolished by PKC inhibitors and reduced by 50% with inhibition of mitogen-activated protein kinase kinase (MEK) (28). In conclusion, muscle contraction and epinephrine both activate HSL through ERK1/2 and PKC signaling pathways during exercise, and HSL activity gradually increases with exercise intensity.

**Regulation of muscle HSL phosphorylation and activity during exercise.** In addition to PKC and MEK kinase/ERK signaling, hormone-sensitive (PKA) and energy-sensing (AMP-activated protein kinase; AMPK) pathways also play an important role in the regulation of HSL phosphorylation and activation. Phosphorylation of HSL Ser⁵⁶³, a PKA target site, was not increased by exercise despite a fourfold increase in plasma epinephrine. This suggests that epinephrine induces HSL activity independently of PKA-induced phosphorylation of Ser⁵⁶³ (100). Increased HSL activity after 1 min of exercise at 65 and 90% VO₂peak was associated with increased Ser⁶⁵⁶ phosphorylation without any changes in Ser⁵⁶³ and Ser⁵⁶⁵ phosphorylation (118). Ser⁵⁶⁵ is a phosphorylation site for AMPK activation, and a role for AMPK has been suggested as well in the regulation of HSL activation. In vitro, AMPK inhibits epinephrine-induced HSL Ser⁶⁵⁶ phosphorylation and activation (135), and HSL activity is reduced in L6 myotubes expressing constitutively active AMPK (133). In vivo, increased α2-AMPK activity in muscle with low glycogen content (achieved by combined dietary and exercise manipulation) is associated with increased HSL Ser⁶⁵⁶ phosphorylation but without any changes in HSL activity and IMTG hydrolysis during submaximal exercise despite higher epinephrine (100). In another study using the same approach, a higher α2-AMPK activity in subjects with low preexercise muscle glycogen content paralleled a reduced HSL activity during exercise (135). However, IMTG content decreased significantly after 1 h of exercise at 70% VO₂peak despite a reduced HSL activity, suggesting the contribution of other lipases and/or factors in the regulation of IMTG hydrolysis during exercise. In conclusion, early HSL Ser⁶⁵⁶ phosphorylation is required for full activation of HSL during exercise. It is still unclear whether increased activity of α2-AMPK during exercise inhibits HSL activity.

**IMTG hydrolysis: a role for gender and training status.** Gender and training status may be important determinants of HSL activity. Skeletal muscle HSL mRNA expression, protein content, and IMTG concentration are higher in women compared with men (Fig. 1); however, HSL total activity is not different between men and women at 90 min of exercise at 60% VO₂peak (99). Roepstorff et al. (99) proposed that a higher IMTG content at baseline might explain the higher net IMTG hydrolysis during exercise in women, i.e., a mass effect. Insulin sensitivity was not measured in this study and could not be related to changes in IMTG content after exercise. In trained vs. untrained subjects, no differences were found in HSL activity and IMTG net hydrolysis before or after 180 min of exercise at 60% VO₂max. Net IMTG hydrolysis tends to be higher in type I fibers in trained subjects (44). Muscle HSL activity must be suppressed during prolonged exercise where the contribution of plasma FFA as fuel for muscle activity rises dramatically (60). Given the known differences in whole body fat oxidation during exercise in trained vs. untrained subjects,
REGULATION OF IMTG BREAKDOWN BY PLASMA FFA AVAILABILITY

Regulation of IMTG breakdown during prolonged exercise. Previous reports have shown that muscle HSL activity is suppressed during prolonged exercise (>1 h), probably because of increased availability of plasma FFA derived from adipose tissue lipolysis. For example, Watt et al. (129) found that, during 120 min of exercise at 60% \( \dot{V}O_{2peak} \), HSL activity increased gradually early on (10 and 60 min) and then decreased to near-resting rates after 120 min. Increased HSL activity at 60 min is consistent with both increased plasma epinephrine and reduced plasma insulin. The decrease in HSL activity during the second hour of exercise was associated with increased muscle LCFA-CoA levels, suggesting that LCFA-CoA may inhibit HSL activity. The increase in muscle LCFA-CoA was tightly associated with a gradual increase in plasma FFA and whole body fat oxidation. Roepstorff et al. (99) and Helge et al. (44) found that exercise >90 min in duration increases the contribution of plasma FFA, again consistent with LCFA-CoA-mediated inhibition of HSL activity and IMTG hydrolysis. The availability and muscle uptake of plasma FFA is likely to be different according to gender and training status, and this could account for differences in IMTG hydrolysis and oxidation. This hypothesis is in line with cross-sectional studies showing a higher lipolytic rate measured by the rate of appearance of glycerol in trained individuals (20) and women (18) compared with untrained individuals and men, respectively, during submaximal exercise practiced at the same relative workload. FFA transporter protein level is higher in women (FAT/CD36) (64) and in trained individuals (fatty acid binding protein membrane; FABPm) (62). Additionally, a higher plasma FFA tissue uptake (45%) during 90 min of exercise at 50% \( \dot{V}O_{2peak} \) has been demonstrated in women (78). However, total fat oxidation during exercise was equal across sex, presumably because of a reciprocal decrease in the oxidation of nonplasma fatty acid sources such as IMTG and plasma triglycerides. Altogether, these results might suggest that increased availability of plasma FFA with long periods of exercise reduces IMTG hydrolysis and oxidation by inhibition of muscle HSL, increasing the contribution of plasma FFA as fuel for exercise. The potential contribution of other TAG lipases in muscle during exercise is an area that should be explored. This is consistent with evidence from the HSL knockout mice showing almost unaffected triglyceride hydrolase activity compared with control animals in skeletal muscle after an overnight fast (42). More recently, Haemmerle et al. (41) have identified a new lipase, adipose triglyceride lipase (ATGL), which plays a major role in the regulation of cellular TAG stores in various tissues of the body including the heart and skeletal muscle. However, the role of ATGL in human adipose tissue seems more limited (72), and no data are presently available on the role of ATGL in the regulation of lipid metabolism in human skeletal muscle.

Influence of peripheral lipolysis on fatty acid oxidation. Another line of evidence that supports the role of FFA availability in the regulation of IMTG hydrolysis and oxidation is derived from in vivo studies using nicotinic acid to suppress peripheral lipolysis. Sustained inhibition of adipose tissue lipolysis and plasma FFA availability during submaximal exercise enhances the oxidation of nonplasma FFA sources including IMTG in lean, healthy, trained subjects (127). This study showed that IMTG content measured specifically in type I fibers was reduced after exercise in the control and nicotinic acid experiments. However, no changes in IMTG content were found in the control and nicotinic acid experiments at rest before exercise. Other studies have shown that higher hydrolysis of IMTG during inhibition of peripheral lipolysis was not associated with changes in muscle HSL or in glycerol-3-phosphate acyltransferase (GPAT) activity (82, 134). This observation again argues for the contribution of other TAG lipases in the hydrolysis of the IMTG pool during exercise. Inhibition of peripheral lipolysis with nicotinic acid during exercise in type 2 diabetic patients increases the contribution of IMTG as substrate fuel for exercise and whole body insulin sensitivity during the postexercise period as measured by the rate of disappearance (\( R_d \)) of glucose (126). IMTG content quantified by Oil Red O staining in type I fibers remained unchanged in the control experiment. This could reflect a blunted capacity for hydrolyzing and oxidizing IMTG in type 2 diabetes patients during exercise. Further experiments are required to better clarify this area. This idea is in line with a study showing impaired \( \beta_2 \)-adrenergic-mediated stimulation of muscle lipolysis in obese subjects (8). Early defects in muscle lipolysis might lead to accumulation of IMTG during obesity and type 2 diabetes. Whether increased insulin sensitivity results from higher depletion of glycogen alone and/or IMTG during the postexercise period needs to be determined. The potential clinical efficacy and safety of such combined exercise and anti-lipolytic therapy to improve insulin sensitivity in type 2 diabetes need to be assessed in randomized clinical trials. These data also suggest that timing of anti-lipolytic therapy might be critical in improving insulin sensitivity.

Influence of dietary fat on IMTG turnover. Consumption of a high-fat diet (HFD) requires increasing fat oxidation to maintain body weight (32). Several studies have shown a reduction of the respiratory exchange ratio (RER) at submaximal workloads of exercise after increasing the fat content of the diet, indicating increased oxidation of fatty acids. Vogt et al. (128) demonstrated that 5 wk of HFD (53% fat) in endurance-trained athletes does not alter muscle glycolgen content but increases IMTG content by twofold. This was accompanied by a decrease in RER at rest and during submaximal exercise. This intervention did not modify mitochondrial volume adjusted per muscle fiber or endurance performance capacity (maximal power and \( \dot{V}O_{2peak} \)). HFD (4 wk)-induced IMTG accumulation may be driven by increased muscle lipoprotein lipase (LPL) activity in men with regular physical training (61). In this study, 1-mo adaptation to a HFD results in increased muscle LPL activity (~2-fold), indicating a higher capacity for uptake of fatty acids from circulating serum triglycerides into the muscle cell in association with a greater capacity for triglyceride storage (1.6-fold) in the muscle. The authors did not notice any changes in plasma insulin, glucose, or triglyceride concentrations after the HFD. Similarly, 2 days of isonenergetic HFD (60% fat) elevated total IMTG content by 36% in endurance-trained cyclists. Moreover, rates of whole body fat oxidation and lipolysis were augmented by 70–80% when the subjects exercised at 50% \( \dot{V}O_{2peak} \) for 1 h (142).
Interestingly, when the same experiment was done during inhibition of peripheral lipolysis by acipimox (a nicotinic acid analog), whole body fat oxidation was slightly reduced but remained twofold higher compared with the control experiment, suggesting a large contribution of “nonplasma” FFA, mainly IMTG, for fat oxidation. Interestingly, the R_d of glucose was not affected overall by the HFD during exercise but was slightly increased under acipimox administration with the control diet. Also, this reveals that skeletal muscle insulin sensitivity is preserved in response to isoenergetic HFD. This suggests that altered substrate storage and increased lipolysis in skeletal muscle is responsible for increased fat oxidation during exercise after short-term HFD. The same kind of diet (60% fat) given for 1 wk to lean, healthy men causes a 54% rise in total IMTG content measured by proton magnetic spectroscopy in vastus lateralis. This metabolic adaptation was associated with an increase in LPL mRNA levels in muscle (108).

Conversely, 2 wk of low-fat (22%) or extremely low-fat diet (2%) provided to endurance-trained cyclists lowers IMTG and proportionally increases glycogen content in muscle. This dietary intervention was accompanied by reduced fat oxidation during 1 h of exercise at 67% \( \dot{V}O_2 \text{max} \), including a compensatory increase in carbohydrate oxidation from glycogen (24). In conclusion, dietary fat increases both IMTG content and oxidation at rest and during submaximal exercise because of reduced glycogen stores in the muscle. The shift of energy metabolism toward lipid as fuel under HFD is considered an important means to spare carbohydrate stores and postpone muscle fatigue. Thus the lowering of muscle glycogen concentration with isoenergetic HFD does not further increase plasma glucose uptake at rest and during exercise. Insulin sensitivity has been shown to be altered mainly with high-energy HFD, which promotes a positive fat balance and an imbalance between lipid storage and fat oxidation (4).

INCORPORATION OF FATTY ACIDS INTO IMTG STORES

FFA uptake. It is now well established that, during fasting conditions, skeletal muscle in lean, healthy individuals relies on lipid oxidation for the majority of resting energy production on the basis of regional indirect calorimetry across the forearm (3, 5) or the leg (55). During postabsorptive conditions, skeletal muscle has a high fractional extraction of FFA and thus is an important site for systemic utilization of fatty acids (55). Some of the FFA taken up by muscle enter tissue lipid pools of triglyceride or phospholipids (31), although it is less clear whether FFA must initially enter tissue triglyceride before oxidation (25) or enter mitochondria directly, as suggested by the isotopic studies of Sidossis et al. (111). Kelley et al. (55) have shown that rates of FFA fractional extraction are similar in lean and obese subjects in fasting conditions in skeletal muscle across the leg. However, arterial FFA concentrations were similar in lean and obese subjects, suggesting equivalent lipid availability to the muscle. Interestingly, the same group reported in another leg balance study a slightly reduced fractional extraction of \([^3]H\)oleate in the skeletal muscle of obese type 2 diabetic subjects but overall higher rates of oleate uptake during the postprandial condition because of a persistent increased arterial FFA concentration (58).

Additionally, skeletal muscle FFA uptake can be regulated by membrane fatty acid transporters including FAT/CD36 (fatty acid translocase), fatty acid transport protein-1 (FATP1), and FABPm. The literature is still controversial on fatty acid transport in skeletal muscle of obese and type 2 diabetic subjects, with studies showing no change (16, 113) or increased (13) fatty acid transporter content. For example, Bonen et al. (13) demonstrated in vitro a fourfold increase in LCFAs transport rates in giant sarcenomembrane vesicles prepared from human skeletal muscle of obese and type 2 diabetic individuals. In a recent study by Pelsers et al. (84), skeletal muscle FAT/CD36 and FATP1 were found to be similarly expressed (both mRNA and protein levels) in sedentary lean and age-matched overweight type 2 diabetic and endurance-trained subjects. FABPm mRNA and protein levels were upregulated by 40% in endurance-trained cyclists compared with type 2 diabetes subjects. This is likely to be an adaptation to allow greater rates of FFA uptake in endurance-trained individuals (84). In summary, skeletal muscle lipid uptake in vivo does not seem to be affected in fasting conditions but is likely increased in obesity and insulin-resistant states during the postprandial phase.

Biochemical pathways and rate-limiting enzymes. TAGs are synthesized from 3 mol of fatty acyl-CoA and 1 mol of glycerol-3-phosphate (G3P). G3P can originate from glycerol through the action of glycerol kinase (GK) or from glucose through the action of glycerol dehydrogenase. The direct conversion of free glycerol to G3P was thought to be negligible in skeletal muscle because of low GK activity measured ex vivo. However, Guo and Jensen (40) have demonstrated, using isotopic tracer dilution techniques, that blood glycerol is a direct and quantitatively important precursor of IMTG synthesis in rat soleus and gastrocnemius, confirming the presence and importance of functional GK in muscle. This observation is supported by in vivo studies in humans showing a significant uptake of glycerol (40%) in the forearm muscle during the postabsorptive state (23). Using human primary skeletal muscle cells, Montell et al. (79) found that \([U-^{14}C]\)glycerol is incorporated into TAG and phospholipids. Adenovirus-mediated overexpression of GK in those cells accelerated the rate of synthesis of TAG and phospholipids. A fraction of \([U-^{14}C]\)glycerol was found to be incorporated in glycogen as well (79). Similarly, overexpression of acyl-GPAT-α (AGPAT-α) in C2C12 myotubes increases the proportion of \([U-^{14}C]\)glucose and \[^{3}H\]oleic acid incorporated into cellular lipids on insulin stimulation (103). More recently, in vivo overexpression of 1,2-acyl-CoA diacylglyceroltransferase-1 (DGAT1) by DNA electroporation in rat tibialis anterior led to an increased accumulation of IMTG in DGAT1-positive myocytes that was further enhanced after a 46% isoenergetic HFD (102). Altogether, those previous experiments reveal that the activities of GK, AGPAT-α, and DGAT1 are rate limiting in the synthesis of IMTG in muscle.

A role for de novo lipogenesis? G3P also originates from glucose through the action of glycerol dehydrogenase. Although de novo lipogenesis has long been thought to occur solely in liver, several studies, mainly in the rat, have shown the incorporation of \([6^{-14}C]\)glucose or \([2,^{14}C]\)acetate into lipids in muscle cells. This effect may be driven by sterol-responsive element binding protein-1c (SREBP-1c), since inhibition of this transcription factor abolishes glucose-mediated upregulation of lipogenic enzymes and incorporation of \([2^{-14}C]\)acetate into the TAG pool (39). De novo lipogenesis...
from glucose has been demonstrated as well in human myotubes exposed to 20 mmol/l glucose for 4 days. Glucose was incorporated into cellular nonesterified fatty acids, TAG, and cholesterol esters, along with an increased DGAT-1 activity (1). It has been demonstrated that incorporation of [U-14C] glucose into total lipids in differentiated human myotubes represents 2–3% of the incorporation of oleate or palmitate into total cellular lipids (34).

Interestingly, myotubes exposed to hyperglycemic medium exhibited a 20% reduction of [14C]palmitate incorporation into cellular lipid, showing the ability of glucose to suppress FFA esterification. Thus, even though obese insulin-resistant subjects exhibit higher expression of muscle acetyl-CoA carboxylase-2 (ACC-2) and fatty acid synthase, the expression of malonyl-CoA decarboxylase remains 40-fold higher than ACC-2, suggesting fatty acid oxidation rather than de novo fatty acid synthesis in muscle (85). However, enzyme mRNA levels are acutely sensitive to changes in metabolic and nutritional signals and are not necessarily correlated with enzyme activities. Thus, it does not exclude the possibility that de novo lipogenesis in muscle may occur during high carbohydrate-rich diets or chronic hyperglycemic conditions such as are encountered during type 2 diabetes. Consistent with this idea, Bandyopadhyay et al. (6) found that increased malonyl-CoA concentration in diabetic muscle might contribute to de novo lipogenesis and TAG synthesis. Additionally, substrate cycling between de novo lipogenesis and lipid oxidation may occur during energy-wasting futile cycle (30). This energy-dissipating futile cycle may provide a fine-tuning mechanism to protect muscle against lipotoxicity. In conclusion, all these studies suggest that de novo lipogenesis in human skeletal muscle could play a role in the increased IMTG accumulation observed in type 2 diabetes.

**IMTG synthesis and insulin sensitivity.** In contrast to oleic acid (C18:1), palmitic acid (C16:0) is poorly incorporated into TAG and induces accumulation of ceramides and apoptosis by activation of caspase-3 (75). A similar study in human primary myoblasts shows that palmitate induces DAG and ceramide accumulation and reduces insulin-stimulated glycogen storage (92). This effect can be almost completely reversed by addition of oleate. Interestingly, both palmitate and oleate are lipotoxic in DGAT1−/− mouse embryonic fibroblasts, used as a model of impaired TAG synthesis. The lipotoxic effect of palmitate can be prevented in Chinese hamster ovary cells by overexpression of stearoyl-CoA desaturase-1 (SCD-1), suggesting a beneficial effect of unsaturated fatty acid (75). Similar results have been recently observed in rat L6 myotubes in which palmitate exposure increases de novo ceramide synthesis, caspase-3 activation, and apoptosis (122). In a recent study by Hulver et al. (48), elevated activity of SCD-1 was associated with disorders in lipid partitioning (defined as the ratio between TAG synthesis and fat oxidation) in muscle tissue obtained from morbidly obese subjects. The overexpression of SCD-1 in muscle cells obtained from lean donors mimicked the obese cellular phenotype, suggesting a role for this enzyme in obesity. One previous report of the potential protective role of SCD-1 against lipotoxicity has been recently confirmed in rat L6 myotubes (93, 122). Targeted inhibition of SCD-1 by short interfering RNA in those cells increases the incorporation of exogenous fatty acids into DAG and ceramides and ultimately impairs insulin-mediated 2-deoxyglucose uptake. This is in line with the results of Haugaard et al. (43), who showed that the improvement in insulin sensitivity during a very low-calorie diet in obese subjects is related to desaturation of both structural (phospholipids) and neutral lipids (IMTG) in muscle. Altogether, these recent studies support the concept that esterification of exogenous FFA into cellular TAG stores may be protective against lipotoxicity and prevent insulin resistance in muscle. In addition, the role of muscle fatty acid desaturase seems to be critical in insulin sensitivity.

Recent studies have shown that TAG accumulation does not seem primarily affected in myotubes from obese and type 2 diabetic subjects exposed to palmitate and oleate (34), suggesting that there is no genetic or intrinsic increase in TAG accumulation. IMTG accumulation would therefore rely on environmental stimuli involving higher FFA availability (as is seen in obesity), FFA uptake due to a passive FFA transport, and reduced oxidative capacity due to decreased mitochondrial content and/or function in obese and type 2 diabetic individuals. IMTG accumulation may be an adaptive mechanism to protect against lipotoxicity and insulin resistance. In support of this concept, overexpression of DGAT1 in mouse skeletal muscle protects mice against HFD-induced insulin resistance by channeling FFA into TAG stores and reducing DAG and ceramide content (76). Similarly, Schenk and Horowitz (106) found that FFA (lipid/heparin)-induced insulin resistance was completely prevented by one session of 90 min of moderate-intensity exercise. This is again supporting the concept of a higher IMTG turnover being protective against insulin resistance. In the setting of lipid infusion performed after a single bout of exercise, we agree with the authors that improvement in lipid partitioning toward IMTG is more critical to improving insulin sensitivity than oxidative capacity. In their study, we calculated that the rate of synthesis of lipid in skeletal muscle was about fourfold higher than the rate of fat oxidation, suggesting the importance of lipid storage capacity for insulin sensitivity in this model.

In conclusion, IMTG accumulation is an adaptive mechanism to buffer excess FFA. Besides improving FFA oxidative capacity, regular physical activity increases muscle lipid storage capacity and increases IMTG stores, which in turn reduce the concentrations of toxic lipid intermediates within the muscle cell. In conclusion, the failure to appropriately sequester FFA into the cellular TAG pool may be a key determinant of insulin resistance.

**CONCLUSION AND FUTURE DIRECTIONS**

IMTG represents a readily available source of energy for skeletal muscle activity during moderate-intensity exercise. IMTG content is high in oxidative type I fibers and in trained subjects in which daily energy turnover is high. IMTG breakdown during exercise requires activation of muscle HSL and is also controlled by endocrine and metabolic signals including FFA availability. Indeed, IMTG breakdown and oxidation are sensitive to acute changes in plasma FFA concentrations achieved either by inhibition of peripheral adipose tissue lipolysis or by dietary interventions. IMTG content is paradoxically increased in sedentary populations including obese and type 2 diabetic individuals.

We propose that there are two different pathways leading to increased IMTG, one a functional adaptation to increased...
energy demand and the other an attempt to buffer a mismatch between lipid supply and oxidation. The latter scenario could be due to increased supply, reduced oxidative capacity, or some combination of the two. Insulin resistance would be driven by accumulation of toxic lipid intermediates, interfering with insulin signaling in muscle. A higher oxidative, lipolytic, and lipid storage capacity and the presence of small lipid droplets in physical contact with mitochondria in the muscle of endurance-trained individuals reflect a higher turnover rate of IMTG. A high turnover rate of the IMTG pool in the fasting condition or during exercise facilitates fat oxidation and is associated with reduced intramyocellular concentration of lipotoxic intermediates such as DAG and ceramides. There are few studies so far that have investigated skeletal muscle lipotoxic intermediate content in endurance-trained subjects compared with lean matched sedentary subjects. Helge et al. (45) attempted to look at skeletal muscle ceramide content and did not find differences in trained vs. untrained individuals. However, the subjects included in the untrained group already had good aerobic capacity (V̇O₂max = 50 ml·min⁻¹·kg⁻¹) and do not represent a true control group. The results would certainly be different with a reference group including subjects with low aerobic potential (V̇O₂max <40 ml·min⁻¹·kg⁻¹), and further studies are required to specifically address this issue. In addition, oxidation is preceded by lipolysis of the IMTG pool, and lipolysis might be a limiting factor of oxidation.

Intramuscular lipolysis is hypothetically well matched to mitochondrial β-oxidation because of the close vicinity of lipid droplets and mitochondria within the myofibrils in endurance-trained individuals. However, a potentially reduced mitochondrial fat oxidation capacity, the presence of bigger lipid droplets positioned away from (without physical contact with) mitochondria, and a low lipid storage capacity in sedentary populations promote a lower turnover rate of IMTG. We propose here that a mismatch between IMTG hydrolysis (lipolysis) and mitochondrial β-oxidation increases the intracellular lipid trafficking with detrimental effects on insulin signaling and glucose metabolism. In conclusion, regular physical activity would be beneficial to protect against FFA-induced insulin resistance in muscle by improving both lipid partitioning toward the IMTG pool and mitochondrial oxidative capacity. Further studies are necessary to clarify the relationship between the rate of turnover of the IMTG pool and the trafficking of lipotoxic intermediates.

ACKNOWLEDGMENTS

This work was supported by USDA Grant 2003-34323-14010 and CNRU NIDDK Grant P30 DK-072476. We are very grateful to Drs. José Galgani and François Crampes for critical reading of the manuscript.

GRANTS

C. Moro was supported by a Lavoisier Postdoctoral Fellowship (Fondation Egide de France).

REFERENCES


Invited Review


