Skeletal Muscle Lipid Metabolism in Exercise and Insulin Resistance

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Kiens, Bente. Skeletal Muscle Lipid Metabolism in Exercise and Insulin Resistance. Physiol Rev 86: 205–243, 2006; doi:10.1152/physrev.00023.2004.—Lipids as fuel for energy provision originate from different sources: albumin-bound long-chain fatty acids (LCFA) in the blood plasma, circulating very-low-density lipoproteins-triacylglycerols (VLDL-TG), fatty acids from triacylglycerol located in the muscle cell (IMTG), and possibly fatty acids liberated from adipose tissue adhering to the muscle cells. The regulation of utilization of the different lipid sources in skeletal muscle during exercise is reviewed, and the influence of diet, training, and gender is discussed. Major points deliberated are the methods utilized to measure uptake and oxidation of LCFA during exercise in humans. The role of the various lipid-binding proteins in transmembrane and cytosolic transport of lipids is considered as well as regulation of lipid entry into the mitochondria, focusing on the putative role of AMP-activated protein kinase (AMPK), acetyl CoA carboxylase (ACC), and carnitine during exercise. The possible contribution to fuel provision during exercise of circulating VLDL-TG as well as the role of IMTG is discussed from a methodological point of view. The contribution of IMTG for energy provision may not be large, covering ~10% of total energy provision during fasting exercise in male subjects, whereas in females, IMTG may cover a larger proportion of energy delivery. Molecular mechanisms involved in breakdown of IMTG during exercise are also considered focusing on hormone-sensitive lipase (HSL). Finally, the role of lipids in development of insulin resistance in skeletal muscle, including possible molecular mechanisms involved, is discussed.
I. PERSPECTIVE

By the end of the 19th century it was suggested by Chauveau and Kaufmann (37) that glucose was the dominating energy substrate during exercise and that lipids were not directly oxidized but first converted into glucose and glycogen in the liver. In those years it was, however, of debate whether glucose was the essential energy substrate for the oxidative metabolism in muscle as Zuntz (345) argued that a mixture of substrates contributed as energy sources.

The carefully conducted experiments by Krogh and Lindhard resolved this debate (165). Krogh’s idea was that the respiratory exchange ratio (RER) measured during identical exercise bouts for 2 h following the ingestion of different diets would indicate the preferential use of substrate for combustion. The main findings of Krogh and Lindhard were 1) that lipids were used as energy substrate and that subjects performed poorly during severe exercise when lipids were the preferential energy fuel, 2) that the preceding diet influenced metabolism during rest and in the postabsorptive state, and 3) that RER values increased with increasing exercise intensities, indicating a greater reliance on carbohydrate as energy fuel.

Subsequently, Christensen and Hansen (40, 41) by measuring oxygen uptake and respiratory quotient further described how diet, training, and exercise intensity and duration affected carbohydrate and lipid utilization.

Today it is well known that lipids, in addition to being necessary as fuel for the organism, have been found to have fundamental roles as messengers and regulators of transcription of genes involved in lipid metabolism. Furthermore, evidence is accumulating that lipids are implicated in the pathogenesis of several common human diseases including the metabolic syndrome, cardiovascular disease, and type 2 diabetes.

Lipids as fuel for energy provision originate from three different sources: albumin-bound long-chain fatty acids (LCFA) in the blood plasma, very-low-density lipoprotein-triacylglycerols (VLDL-TG), fatty acids from triacylglycerol located in the muscle cell (IMTG), and possibly fatty acids liberated from adipose tissue adhering to the muscle cells. This review focuses on the role of lipids in fuel provision in skeletal muscle during exercise and in insulin resistance of skeletal muscle.

II. LONG-CHAIN FATTY ACID UTILIZATION DURING EXERCISE

At the onset of exercise, there is a large increase in uptake and oxidation of LCFA in skeletal muscle. It is also well recognized that prolonged exercise for several hours at a low intensity induces a gradual decrease in the respiratory quotient and hence an enhanced lipid utilization at the expense of carbohydrates as energy fuel. Moreover, when the exercise intensity increases, a shift in fuel selection appears towards an increase in carbohydrate and decrease in fat utilization, and following endurance training a shift towards an enhanced lipid utilization is evident at least when exercise is performed at the same absolute work load in the untrained and trained state. It is interesting to note, however, that this training-induced shift in fuel selection is prevented when a carbohydrate-rich diet is consumed (116, 119). Even though a change in lipid utilization under different conditions is well described, the regulatory mechanisms controlling fatty acid uptake and oxidation are not yet completely known.

A. Arterial Long-Chain Fatty Acid Concentration

The level of the arterial concentration of LCFA during exercise is dependent on the preexercise diet, on the time elapsed since the last meal (73, 197), and on whether carbohydrates are consumed during exercise (194, 200). In general, the higher the fat content of the preexercise diet and the longer after the last meal, the higher the concentration of LCFA in plasma, whereas carbohydrate feeding inhibits the exercise-induced increase in arterial LCFA concentration due to an increase in arterial plasma insulin concentration (194) and consequently a decrease in adipose tissue lipolysis (129). During exercise an initial decline in the LCFA concentration is often observed followed by a slow increase. Therefore, if exercise is prolonged, the arterial plasma concentration of LCFA may increase to values markedly above resting levels, but at the most to resting levels during more intense exercise (3, 30, 35, 78, 79, 162, 239, 300). The initial drop in arterial LCFA concentration during exercise is most likely caused by an imbalance between slow mobilization of fatty acids from adipose tissue and rapidly increased extraction of LCFA by skeletal muscle.

B. Utilization of Long-Chain Fatty Acids During Exercise

Whole body LCFA uptake during exercise can be measured by tracer techniques, infusing either radioactive or stable isotopes. Combining tracer data and indirect calorimetry, findings generally indicate that ~55–65% of total whole body fat utilization during moderate-intensity exercise is derived from plasma fatty acids (79, 117, 235, 239, 312). During prolonged submaximal exercise, the contribution of LCFA to energy provision increases with time (3, 314). If palmitate is labeled on the carbon molecules ($^{14}$C or $^{13}$C), it is also possible to measure LCFA oxidation, provided that the bicarbonate pool has been prelabeled. Experiments have been conducted using infusion of $^{13}$C- or $^{14}$C-labeled acetate to correct for the...
amount of labeled carbon lost in side reactions in the tricarboxylic acid (TCA) cycle or fixed in the bicarbonate pool following oxidation of labeled LCFA. Different whole body acetate recovery values have appeared in the literature as this value obviously depends on exercise intensity and the location of the labeling on acetate. Accordingly, the whole body acetate recovery values obtained during exercise varied from 66 to 94% at exercise intensities ranging from 23 to 84% of maximal oxygen consumption when $^{1-13}$C-labeled acetate or $[1-13]$C]acetate was infused (268, 296). When $[1,2-13]$C]acetate was infused during bicycle exercise, whole body recovery ranged from 69 to 100% at exercise intensities from 40 to 75% of maximal oxygen consumption (237, 259, 313). The highest values were measured in the trials with the highest exercise intensity. During knee-extensor exercise during which only 2–3 kg muscle is engaged in the exercise, the whole body recovery of infused $[1,2-13]$C]acetate was $\sim$85% (309). It is interesting to note that when the label is on the 2-position of acetate only, recovery is markedly less (296) and not representative for either 1-C or $[U-13]$C]palmitate due to the higher tendency of the 2-carbon of acetate to participate in exchange reactions in the TCA cycle compared with the 1-carbon (339). When measurements are performed using arteriovenous leg balance methods during exercise, acetate recovery was close to 100% (237, 309).

When applying correction factors obtained by $[1,2-13]$C]acetate, the available investigations utilizing infusion of $[U-13]$C]palmitate demonstrate that 80–96% of whole body LCFA rate of disappearance ($R_d$) were oxidized during bicycle exercise at intensities ranging from 40 to 75% of maximal oxygen uptake (235, 312, 314). In one study there was a clear trend towards the highest percentage oxidation values at the highest work loads (312). It was also shown that when preexercise muscle glycogen content is low and fat oxidation during exercise consequently is high, the percentage of whole body LCFA $R_d$, which is oxidized is higher than when preexercise glycogen content is high, suggesting an influence of glycogen stores on oxidation of the LCFA taken up (237).

When $[1-13]$C]palmitate was infused, ~70% of the systemic plasma LCFA was oxidized during exercise at 68% of maximal oxygen uptake (117) when a mean acetate correction factor suitable for $[1-13]$C]palmitate was applied. In studies in trained and untrained men, 58 and 76% of $[1-13]$C]oleate were oxidized during exercise, representing 40 and 80% of peak oxygen uptake, respectively, also using a mean acetate correction factor suitable for $[1-13]$C]oleate (269). The lower percentage of plasma LCFA oxidized in these studies obtained with 1-C than with U-C-labeled tracers is probably due to loss of label inside reactions when using 1-C tracers, and all together, these observations tend to indicate that uniformly labeled tracers are preferable to 1-C-labeled tracers.

Concluding from the available evidence, it is evident that a major part of plasma LCFA taken up during exercise is oxidized in the body, and the fate of the remaining LCFA is likely to be esterification in noncontracting muscles or other tissues not directly involved in the exercise. In agreement with this assumption, it has been reported in humans that muscle contraction diverts leg uptake of LCFA to oxidation rather than to reesterification in IMTG (241) and that during leg exercise nonexercising muscle may in fact take up and reesterify LCFA to IMTG (262). Along these lines, it is also possible that esterification takes place in the nonactive motor units in a muscle working at a submaximal exercise intensity, whereas esterification of LCFA in the actual contracting fibers probably is less likely.

An important question is, however, the extent to which data obtained in whole body measurements reflect what is occurring locally in the exercising muscle. This question is obviously mostly relevant when large muscle groups are involved in exercise such as in bicycle ergometer exercise. To address this question, it is necessary to simultaneously measure whole body and leg uptake and oxidation of LCFA. Our own studies at 60–68% of peak pulmonary oxygen uptake show that only 32–45% of the systemic plasma LCFA total uptake ($R_d$) occurred in the two legs (117, 235, 237). Supplementing these data, the study by Burguera et al. (30) showed that during exercise at 45% of maximal oxygen consumption the uptake in the legs was ~60% of whole body LCFA turnover during exercise. That such a relatively small fraction of systemic plasma LCFA total uptake is extracted in the active muscles implies that about one-half of the systemic plasma LCFA presumably is taken up in adipose tissue, the heart, liver, inactive or slightly active upper body muscles, and possibly other organs during bicycle exercise. However, looking at LCFA utilization at rest and during exercise, it appears that at rest there is a relatively high whole body lipid turnover, and the increase with submaximal bicycle exercise is about two- to threefold (30, 117, 235, 237). In contrast, leg LCFA uptake at rest is very low and the increase with exercise is on the order of 5- to 15-fold (30, 235). Thus another way to compare whole body and leg LCFA uptake is to compare the increase in utilization with exercise (Table 1). With an examination of data from the study by Roepstorff et al. (235), a gender comparison is also possible. It appears that 60–76% in endurance-trained females and males, respectively, of the exercise-induced increase in whole body LCFA $R_d$ can be accounted for by uptake in the legs during submaximal bicycle exercise at 60% peak oxygen uptake, and no major gender effect was demonstrated in these endurance-trained individuals (Table 1). Thus 24–40% of LCFA $R_d$ is taken up elsewhere than the exercising muscles. During one-legged exercise in which only a relatively small muscle mass (2–3 kg) is engaged in exercise, ~55% of the increase in whole body...
TABLE 1. Exercise-induced increase (Δ) in mean whole body and leg uptake of LCFA and percentage of increase in whole body uptake of LCFA that occurs across the legs

<table>
<thead>
<tr>
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<th>Females</th>
<th>Males</th>
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<tr>
<td>Δ WB FA uptake, μmol/min</td>
<td>600</td>
<td>500</td>
</tr>
<tr>
<td>Δ Leg FA uptake, μmol/min</td>
<td>360</td>
<td>380</td>
</tr>
<tr>
<td>Δ Leg/ΔWB, %</td>
<td>60</td>
<td>76</td>
</tr>
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WB, whole body; FA, fatty acid; LCFA, long-chain fatty acid. [Data from Roepstorff et al. (235) and C. Roepstorff and B. Kiens, unpublished data.]

palmitate oxidation was accounted for by increase in leg palmitate oxidation (310). Collectively the data on whole body and leg uptake of LCFA indicate that because of the relatively high whole body resting uptake (Rd) of LCFA, the Rd of LCFA during exercise only to a limited extent reflects uptake of LCFA in active muscle during exercise.

It is worthwhile to note that due to simultaneous uptake and release of LCFA, true tracer-determined LCFA uptake is two to three times higher than net uptake (101, 117, 235, 300). In this respect, it might also be worth noting that while it has been suggested, based on experiments in three subjects, that net leg LCFA uptake is underestimated when the femoral venous catheter is placed in the normal antegrade (pointing towards the heart) direction rather than the retrograde direction (pointing towards the knee), tracer-derived leg LCFA uptake is not affected by catheter placement (310).

In experiments where leg uptake and oxidation of plasma LCFA is determined, it is also possible to measure how much of the LCFA taken up across the legs was directly oxidized. Values ranging from 72 to 100% of tracer-derived LCFA uptake in the legs during exercise have been reported, and they vary with gender (235), preexercise muscle glycogen stores (237), and exercise mode (310). These values are within the relative wide span of values obtained in older studies, revealing that 60–100% of radioactive 14C-labeled palmitate or oleate taken up in plasma LCFA turnover occurs in the exercising muscle. On the other hand, the exercise-induced increase in leg uptake of LCFA reflects the measured exercise-induced increase in whole body Rd fairly well, although the former underestimates the latter to some extent. The underestimation may be ascribed to LCFA uptake in accessory exercising muscle other than the legs. Moreover, it appears that the higher the exercise intensity, the closer to 100% oxidation of leg uptake of LCFA is achieved, whereas at low exercise intensities, an incorporation of the LCFA into IMTG may occur probably in inactive motor units.

III. REGULATION OF LONG-CHAIN FATTY ACID UTILIZATION IN SKELETAL MUSCLE DURING EXERCISE

A. Supply of Long-Chain Fatty Acids

There are several steps involved in the pathway from LCFA release either from adipose tissue or from the triacylglycerol-rich lipoproteins in the circulation into their final oxidation in the mitochondria which could play a significant role in regulation of LCFA oxidation (Fig. 1).

Earlier studies pointed to the uptake and oxidation of LCFA being largely determined by the rate of lipolysis in adipose tissue as a linear relationship was usually described between LCFA plasma concentration and the rate of plasma LCFA uptake and oxidation (3, 100, 102, 107, 209). To evaluate lipid metabolism more specifically in a well-defined human muscle group (5), a series of human experiments were performed in which exercise was allocated to the knee-extensors of the thigh. Subjects exercised-trained the knee-extensors of one thigh for 8 wk while the knee-extensors of the other leg served as control. Then acute prolonged exercise was performed with the trained knee-extensors 1 day and with the untrained control knee-extensors another day, in a randomized order (147). Despite a continuous exercise-induced increase in plasma LCFA concentration, uptake of LCFA in the untrained thigh only increased initially during exercise and then leveled off, whereas when subjects exercised with the trained thigh at the same absolute work load as in the untrained thigh, a continuous increase in uptake of LCFA occurred (147). Also, when untrained and trained subjects performed dynamic knee-extensions with one leg at the same relative work load, palmitate uptake and oxidation in the knee-extensors leveled off with time in the untrained subjects but continued to increase in the trained subjects.
(300) (Fig. 2). Similarly, in the isolated, perfused rat skeletal muscle, palmitate uptake at rest (298) and during contractions (299) displayed saturation kinetics when plotted against the unbound perfusate palmitate concentration. The studies lend weight to the idea that the arterial concentration of LCFA to some extent is of importance for extraction of LCFA in contracting muscle, but it is likely that other factors inherent in the muscle are of importance too.

FIG. 1. Schematic representation of the likely routes taken by fatty acids from the capillary to the mitochondria. VLDL, very-low-density lipoprotein; ALB<sub>F</sub>, albumin bound fatty acid; LPL, lipoprotein lipase; ALB<sub>B</sub>, albumin receptor; LBP, lipid binding protein; ACS, acyl CoA synthetase; FABPC, cytosolic fatty acid binding protein; ACBP, acyl CoA binding protein; TG, triacylglycerol; LCFA, long-chain fatty acids; CD36, fatty acid translocase; FABP<sub>mem</sub>, membrane fatty acid binding protein.

FIG. 2. Arterial plasma concentration (A), fractional uptake (B), uptake (C), and total oxidation (D) of free fatty acids (FFA = long-chain fatty acids) across the thigh during rest and 3 h of knee extension exercise in trained and untrained subjects. Values are means ± SE of 6 subjects in trained and untrained groups. *P < 0.05 compared with untrained; †P < 0.05 compared with previous value. [From Turcotte et al. (300).]
Further support for this notion is found in the study by Romijn et al. (240). In their study intralipid plus heparin was infused during exercise to increase the concentration of LCFA in plasma. During exercise at 85% of maximal oxygen uptake, the uptake of LCFA was only 27% higher in the intralipid trial compared with the control trial, even though the plasma LCFA concentration averaged 2.13 mM in the intralipid trial in contrast to 0.29 mM in the control trial.

B. Transport From Circulation to Cytosol

From the circulation LCFA must pass the endothelium, the interstitial space, the plasma membrane, the cytosol, and the mitochondrial membranes for their final oxidation in the mitochondria (Fig. 1). The elaborate chain of reactions provides a number of possible points of regulating the supply of LCFA for oxidation. It has been a matter of debate whether the transendothelial and/or transsarcolemma transport of LCFA is a passive process dependent on the rate of cellular metabolism or occurs via plasma membrane protein-mediated transport. However, from recent studies it seems that both mechanisms are operating (22, 219).

Within recent years three putative fatty acid binding proteins located at the plasma membrane have been identified. These are 1) the plasma membrane-bound fatty acid binding protein (FABP<sub>pm</sub>), 2) fatty acid translocase (FAT/CD36), and 3) the fatty acid transport protein (FATP). FABP<sub>pm</sub> is a ~43-kDa protein located peripherally on the plasma membrane, which in fact is identical to the mitochondrial enzyme aspartate aminotransferase (mAAT) (26, 288). FAT/CD36 is a 88-kDa integral membrane glycoprotein with two predicted transmembrane domains, and it is found to be 85% homologous to the glycoprotein IV or CD36 of human blood platelets and leukocytes (2). FATP is a 63-kDa integral protein with six predicted transmembrane domains (253), and recently a family of FATPs was identified (122). Both FABP<sub>pm</sub> and FAT/CD36 are present in most metabolic tissues including human skeletal muscle (24, 31, 32, 149, 155, 234) (Fig. 3), and the transcript of FATP1 mRNA has also been detected in human muscle (24, 155). To date, the mechanism of action of these lipid binding proteins is not well known, but increasing evidence is emerging that they are involved in fatty acid uptake.

1. FAT/CD36

Recent evidence supports an important role for FAT/CD36 in uptake of LCFA in rodent skeletal muscle. For instance, overexpression of FAT/CD36 in mice reduced plasma triacylglycerol and LCFA concentrations and increased palmitate oxidation in contracting soleus muscle compared with wild-type muscles (135). Conversely, FA uptake was reduced in FAT/CD36 null mice (71). When plasma membranes were isolated, using the giant vesicle preparation, a higher FAT/CD36 protein expression was obtained in muscles from streptozotocin-induced diabetic rats (181) and in muscles from obese Zucker rats (182), which display increased fatty acid transport. Conversely, when rats were treated with leptin for 14 days, a reduction was obtained in sarcolemmal FAT/CD36 protein content in association with a decrease in FA transport (282). In humans, a fat-rich diet induced an increase in the expression of FAT/CD36 measured in homogenates from the vastus lateralis muscle (32, 238).

Both triacylglycerol content and FAT/CD36 protein and gene expression measured in the vastus lateralis muscle appeared to be higher in women than in men (155, 281) (Fig. 4). Furthermore, an increase in triacylglycerol storage was found in skeletal muscle (117, 148) in concert with an increase in FAT/CD36 protein induced by a high-fat diet (238). Moreover, in obese and type 2 diabetic men and women, the amount of FAT/CD36 protein, measured in giant vesicles prepared from the rectus abdominus muscle, was higher compared with lean controls, and the FAT/CD36 protein expression correlated with the IMTG content (25). On the basis of these findings of simultaneous high expression of FAT/CD36 and IMTG content, it could be speculated that an enhanced amount of FAT/CD36 would be of benefit for clearance of circulating LCFA. Once taken up in the skeletal muscle, the LCFA could be either oxidized in the mitochondria or directed towards resynthesis into triacylglycerol. If the need for energy is low compared with the uptake of LCFA, the latter will be directed towards resynthesis into IMTG. In

![Fig. 3: Representative Western blots of fatty acid translocase (FAT)/CD36, plasma membrane-bound fatty acid binding protein (FABP<sub>pm</sub>), cytosolic fatty acid binding protein (FABP<sub>c</sub>), and acyl CoA binding protein (ACBP) in human skeletal muscle total crude membranes (FAT/CD36, FABP<sub>pm</sub> and ACBP) or cytosolic (FABP<sub>c</sub>) fractions showing a single band at ~88, 43, 15, and 10 kDa, respectively.](http://physrev.physiology.org/)

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accompanying with this suggestion, overexpression of FAT/CD36 in C2C12 myotubes increased intracellular triacylglycerol content in the presence of excess (750 μM) palmitate but not when palmitate in the medium was lowered (80 μM) (11).

The localization of FAT/CD36 in human skeletal muscle was recently investigated morphologically (143, 320) (Fig. 5). In cryosections from the vastus lateralis muscle, immunofluorescently labeled with anti-FAT/CD36, it was shown by confocal microscopy that FAT/CD36 was highly expressed in endothelial cells and to a lesser degree in the sarcolemma (143, 320). From these studies it also appeared that FAT/CD36 was more abundant in type 1 than type 2 muscle fibers (143, 320). These findings are in agreement with morphological studies in rat (343) and equine muscle (306).

Interestingly, while it was found that FAT/CD36 was associated with the plasma and endothelial membrane, no staining of FAT/CD36 was seen in intracellular compartments (320, 343), whereas others reported that intracellular clusters of FAT/CD36 could be identified in type 1 fibers only (143, 306). Even so, the intracellular labeling was very weak and not associated with mitochondria (143). That FAT/CD36 was localized to the sarcolemma with no apparent staining in the cytosol seems to be in contrast to findings from fractionation studies in rat skeletal and heart muscle where FAT/CD36 was detected in both an intracellular depot and in the plasma membrane and that translocation between these two pools can take place in the resting state and upon various stimuli such as insulin, leptin, AICAR, and electric stimulation (23, 183, 185, 188, 282) and hence acutely regulate LCFA uptake. However, detection of intracellular FAT/CD36 may also be due to processing of newly synthesized proteins on their way to the plasma membrane rather than mature protein sequestered in a storage compartment (4). On the other hand, a FAT/CD36 pool located beneath and in close association with plasma membrane cannot be excluded from the morphological studies. Recently, by subcellular fractionation, the presence of FAT/CD36 protein expression was demonstrated in subsarcolemmal and intramyofibrillar located mitochondria in white and red gastrocnemius muscle of female rats (33). Considering the immunohistochemical data, this finding is surprising but may possibly indicate that FAT/CD36 located in the mitochondria may have the epitope, against which the antibody is raised, buried in the mitochondrial membrane, and that the epitope is only exposed when membrane structures are degraded during lysate production for Western blot.

2. FABP

With regard to FABP pm, this protein may also play a role in the transport of LCFA across the sarcolemma. In rat muscle, fasting (48 h) increased FABP pm protein content, but only in oxidative muscles which are highly dependent on fatty acid metabolism (301). A role for FABP pm in transport of long-chain saturated and unsaturated fatty acids has been suggested from use of antibodies against FABP ps, which leads to inhibition of LCFA uptake in various cell types and of transport of LCFA into skeletal muscle giant vesicles in rats in a dose-dependent manner (263, 274, 287, 303, 344). Similarly, overexpression of FABP pm by transfection in rat skeletal muscle increased fatty acid transport and metabolism in resting muscle (42). However, elucidation of the role of FABP pm in LCFA transport was challenged by the findings that FABP pm is identical to the mitochondrial isoform of the enzyme aspartate aminotransferase (mAspAT) (26, 288). In favor of a role of mAspAT/FABP ps in fatty acid binding, molecular modeling studies of the crystal structure of mAspAT have identified a pocket, within the larger domain of the enzyme, which is of sufficient size to accommodate the typical LCFA (15). Whether in fact this pocket serves as a fatty acid binding site remains to be elucidated. Recently, a study using a polyclonal antibody against rat mAspAT in immunogold electron microscopy of rat tissue sections has reported a strong labeling of mitochondria in several cell types (36). Labeling was also observed in other locations such as endothelial cell surfaces, and it was concluded from these observations that mAspAT/FABP pm is both a mitochondrial enzyme and a plasma membrane protein (36).

Dietary manipulations affect FABP pm protein expression. A high-fat diet induced an increase in FABP pm protein expression in vastus lateralis muscle in male volunteers, and a carbohydrate-rich diet decreased the FABP pm content (238). The upregulation of FABP pm protein was however only obtained after long-term dietary interventions as short-term dietary interventions did not affect FABP pm protein expression (32, 238). In human obesity, a
higher FABP<sub>pm</sub> protein expression in homogenates from the vastus lateralis muscle was found compared with lean subjects, irrespective of gender (271). This is in contrast to findings by Bonen et al. (25), who showed a reduced or no difference in protein expression of FABP<sub>pm</sub> measured in homogenates or plasma membranes (from giant vesicle preparations), respectively, from the rectus abdominis muscle in a gender mixture of obese compared with lean subjects. The discrepancy between these findings might be ascribed to the fact that different muscles have been investigated.

Exercise training is another condition where the capacity for lipid metabolism is changed. Accordingly, a higher FABP<sub>pm</sub> protein expression in homogenates from the vastus lateralis muscle was also demonstrated with exercise training (149, 155). The training-induced upregulation of FABP<sub>pm</sub> protein is obviously related to gender as changes in FAPB<sub>pm</sub> protein were not obtained in women (155). Interestingly, gender differences in FABP<sub>pm</sub> protein content in the vastus lateralis muscle were not obtained in nontrained subjects (155, 271) in contrast to findings of FAT/CD36 protein content, which is higher in females than in males (155).

3. FATP1

FATP1 is expressed in adipose tissue, heart, and skeletal muscle of mouse and rat (1, 122, 250, 253) and in skeletal muscle of humans (24, 155). Evidence for the importance of FATP in LCFA transport comes from experiments in cultured cells (122, 253) and yeast (67). A number of studies have reported that FATP1 has acyl CoA synthase activity (45, 105, 322), indicating a role in conversion of fatty acids to fatty acid acyl CoA. In growing
293 cells, fatty acids taken up through FATP1 were preferentially channeled into triacylglycerol synthesis, which has suggested a functional link between FATP1-mediated fatty acid uptake and lipid storage (106). Further support for this contention are the findings in FATP1 knock-out mice of a marked reduction in triacylglycerol and diacylglycerol content in the quadriceps muscle after 3 wk high-fat diet compared with wild-type mice (158). FATP1 protein content was markedly higher in homogenates from the soleus than the gastrocnemius muscle in rats (192), and high-fat diets caused an elevation in FATP1 protein content in the soleus muscle, but a reduction in the gastrocnemius muscle. In a group of matched females and males, vastus lateralis muscle FATP1 mRNA expression was not different despite the findings of significantly higher IMTG in the females than in the males (155, 281).

4. Caveolins

Within the recent years caveolae and lipid rafts have been suggested to be involved in fatty acid uptake. Caveolae are 50- to 100-nm flask-shaped invaginations of the plasma membrane. Caveolae have been well described in adipocytes, in endothelial cells, in type 1 pneumocytes of the lung, and in skeletal muscle cells. Caveolae are a morphological subclass of lipid rafts, specialized microdomains, composed of sphingolipids and cholesterol which contain caveolin, the protein which is essential for invagination of the plasma membrane (for review, see Ref. 46). Three caveolins have been identified of which caveolin-1 and caveolin-2 are expressed in most cell types, whereas caveolin-3 is restricted to skeletal muscle (254, 293).

Recent data in HepG2 and endothelial cells suggest that caveolae may play a significant role in uptake and intracellular trafficking of LCFA (217, 231). With regard to caveolin-1, it was shown that detergent-insoluble fractions from alveolar type II cells were enriched in FAT/CD36 and caveolin-1 (163) and furthermore that caveolin-1 binding of fatty acids was saturable (295). In the caveolin-1 null mice, it was found that serum triacylglycerol and fatty acid concentrations were markedly increased (293). Within the recent years caveolae and lipid rafts have been suggested to be involved in fatty acid uptake. Caveolae are 50- to 100-nm flask-shaped invaginations of the plasma membrane. Caveolae have been well described in adipocytes, in endothelial cells, in type 1 pneumocytes of the lung, and in skeletal muscle cells. Caveolae are a morphological subclass of lipid rafts, specialized microdomains, composed of sphingolipids and cholesterol which contain caveolin, the protein which is essential for invagination of the plasma membrane (for review, see Ref. 46). Three caveolins have been identified of which caveolin-1 and caveolin-2 are expressed in most cell types, whereas caveolin-3 is restricted to skeletal muscle (254, 293).

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Besides the indications of an association of caveolin-1 and FAT/CD36 in human skeletal muscle endothelial cells, we also recently showed, in longitudinal muscle sections, in which part of a fiber was cut tangentially, a high degree of colocalization of caveolin-3 and FAT/CD36 in the sarcolemma (320) (Fig. 5). One putative mechanism whereby caveolin could be involved in cellular fatty acid uptake is that caveolae may regulate the function of fatty acid transporters such as FAT/CD36.

The endothelial cells lining the capillary are the first transport barrier for the circulating fatty acids before transport into muscle cell. Earlier studies suggested that a specific interaction of the albumin-FA complex with proteins associated with the endothelial membrane facilitated the dissociation of the FA-albumin complex and, hence, the transfer of FA from the vascular to the interstitial compartment (307). Because FAT/CD36 is present abundantly in the capillary endothelium as well as in the sarcolemma, FAT/CD36 may play a role in the transport both in the endothelial cells and in the plasma membrane. Because the protein has adhesion functions (72), it may be speculated that FAT/CD36 in the endothelium could be involved in adherence not only of albumin-bound fatty acids but also of fatty acids released from the hydrolysis of circulating VLDL-TG by lipoprotein lipase activity.

Summarizing the available data, it would seem that the fatty acid binding proteins expressed in the endothelium and plasma membrane are involved in fatty acid uptake and that caveolae and caveolins may be involved in this process as well. Yet, the precise functional role of and mechanisms by which the proteins are mediating LCFA uptake is not known, and the mechanisms of LCFA uptake into various mammalian cells may not be the same. It may seem redundant that muscle cells express several types of fatty acid transporters, but the possibility exists that they have distinct roles in the transport process, or that they function in a coordinated manner in the transport of LCFA. However, this is not known at present.

5. Influence of exercise

Then the question arises whether the transport proteins may play any regulatory role in LCFA uptake and oxidation in human skeletal muscle during exercise, a situation where LCFA uptake and oxidation can increase manyfold. In rat skeletal muscle, uptake of palmitate into sarcosomal giant vesicles increased 50–75% after contractions (23) and correlated with expression of FAT/CD36 (187). The training-induced increase in LCFA utili-
zation during exercise (147, 300) (Fig. 2) could theoretically be ascribed to an increased number and/or activity of the lipid-binding proteins in the trained compared with the nontrained state, which might facilitate LCFA transport into the myocyte. This view was supported by the findings of an increase in muscle FABP<sub>pm</sub> protein expression with endurance exercise training in male subjects (149, 155). In addition, training of rats led to an increase in muscle FABP<sub>pm</sub> together with a contraction-induced uptake and oxidation of palmitate (302). The training-induced increase in FABP<sub>pm</sub> is apparently gender specific because the FABP<sub>pm</sub> protein content was similar in untrained and well-trained female volunteers (155). Interestingly, when comparing endurance-trained and untrained female and male subjects, FAT/CD36 protein content in vastus lateralis muscle was not different (155). In contrast, short-term training for 9 days (297) as well as even a single exercise bout (237) increased FAT/CD36 protein content slightly (20–25%) in muscle, perhaps suggesting that increased FAT/CD36 expression is an early adaptation to increased muscle activity that may wane with sustained increased activity.

Even though there is evidence that the fatty acid binding proteins are involved in transport of LCFA across the membrane, this does not necessarily mean that the transport proteins or the transport process is rate limiting for fatty acid utilization during exercise. On the one hand, overexpression of FAT/CD36 increases fatty acid oxidation during electrically induced muscle contractions (135), supporting a regulatory role of FAT/CD36. On the other hand, an intracellular accumulation of fatty acids was demonstrated in human vastus lateralis muscle when exercise intensity was increased from 65 to 90% of VO<sub>2peak</sub> and fat oxidation decreased markedly (154). This increase in LCFA content within the muscle cell during the intense exercise was even found in parallel with a significant decrease in blood plasma LCFA concentration (154). These findings give support to the notion that when exercise intensity is increased and fat oxidation decreases, fatty acid oxidation is limited by factors inside the muscle cell, rather than by limitations in transmembrane transport. In addition, by manipulating the preexercise muscle glycogen levels, Roepstorff et al. (237) achieved one situation with high fat oxidation and another with high carbohydrate oxidation in human skeletal muscle during a submaximal bicycle exercise bout. This resulted in a 100% higher leg plasma LCFA oxidation, measured by the tracer technique, during the high fat-oxidation trial compared with the high carbohydrate-oxidation trial, but the leg uptake, clearance, and fractional extraction of circulating LCFA were similar in the two trials. These findings further strengthen the view that there are several exercise conditions during which transmembrane transport during exercise may not be the important limiting step in plasma LCFA oxidation.

C. Transport in the Cytosol

LCFA taken into cells are activated in the cytosol by reaction with CoA and ATP to yield long-chain fatty acyl CoA (LCFA-CoA) catalyzed by long-chain acyl CoA synthetase (ACS) (Fig. 1). In addition to being substrates for β-oxidation and triacylglycerol synthesis (48), it has also been proposed that LCFA CoA esters play a role in enzyme activation (68, 180), vesicular trafficking (273), and cell signaling (28). The active site of ACS has been located to the cytosolic surface of the peroxisomal endoplasmic reticulum and outer mitochondrial membranes (48). It was recently demonstrated in 3T3-L1 adipocytes that long-chain ACS is an integral membrane protein also located in the plasma membrane (84), and it was suggested that incoming LCFAs are immediately esterified at the plasma membrane. An efficient esterification maintains a low intracellular LCFA concentration and contributes to uptake of LCFA. ACS may therefore affect both the rate and directionality of LCFA movement across membranes and may be coupled to other proteins that participate in LCFA uptake such as FATP 1, which was reported to have ACS activity (45, 84, 105, 252, 322). There is now strong evidence that the intracellular transport of LCFA moieties is mediated by a cytoplasmic fatty acid binding protein (FABP<sub>c</sub>) (17, 86, 88) and a cytoplasmic acyl CoA binding protein (ACBP) (226). Very little LCFA and LCFA-CoA actually exist as free or unbound molecules but are rather bound to cytosolic FABP<sub>c</sub> and ACBP. LCFA-CoAs are amphipathic molecules and bind strongly to phospholipid membranes. Data indicate that the cytosolic binding proteins act in extracting LCFA-CoA from and prevent their binding to biological membranes and liposomes and donate the LCFA-CoAs for metabolic β-oxidation (87, 226). Even though LCFA-CoAs are relatively water soluble and might move through the cytosol in an unbound fashion, their binding to FABP<sub>c</sub> and ACBP is believed to be a major factor in controlling the free concentration of cytosolic LCFA-CoA. The importance of FABP<sub>c</sub> in LCFA uptake was revealed in mice, where the gene of the heart isoform of FABP<sub>c</sub> was ablated. This resulted in a reduced uptake of LCFA in heart of ~50% (199). In skeletal muscle, uptake of LCFA was decreased by ~45% in homozygous mice but was unaffected in heterozygous mice (184).

Other conditions altering the content and regulation of the cytosolic fatty acid binding proteins are interventions leading to changes in lipid metabolism. Dietary manipulation is one such intervention. Available information is mainly on the cytoplasmic FABP<sub>c</sub>. Moreover, most data are from rat studies, where animals have been fed a fat-rich diet, mainly composed of saturated fatty acids (44). Collectively, these data have shown that FABP<sub>c</sub> in heart and skeletal muscle did not respond to an increase in saturated dietary fatty acids (44, 285). In contrast, a study in rat heart and skeletal muscle revealed that inges-
tion of a diet rich in omega-3 fatty acids markedly increased FABP<sub>c</sub> content (43), suggesting that the length and degree of saturation of the carbon chain of the fatty acids are important for regulation of FABP<sub>c</sub>. In agreement with these findings, in humans the ingestion of a fat-rich diet in which the ratio of polyunsaturated to saturated fatty acids was relatively high (0.6) induced a significant increase in FABP<sub>c</sub> protein content in the vastus lateralis muscle, in contrast to the ingestion of carbohydrate-rich diet for 4 wk (238). It has been suggested that FABP<sub>c</sub> cooperates with the membrane-associated lipid binding protein FAT/CD36 in uptake of LCFA in cardiac and skeletal muscle (186). Interestingly, in a dietary intervention study, a similar increase in FABP<sub>c</sub> and FAT/CD36 protein content was observed, in the vastus lateralis muscle following a fat-rich diet in human volunteers in contrast to when a carbohydrate-rich diet was consumed (238).

Even though exercise training increases the potential for a higher lipid oxidation, neither FABP<sub>c</sub> nor ACBP levels in vastus lateralis muscle in humans were changed by exercise training, suggesting that the amounts of these cytosolic proteins were abundant and sufficient in trafficking LCFA-CoA esters and LCFA within the cell during exercise (155). These data are supported by recent findings in rats showing that endurance training did not affect the protein level of ACBP in skeletal muscle (74) even though the lipid oxidation potential in muscle was increased.

D. Metabolism in Mitochondria

Long-chain fatty acyl CoA (LCFA-CoA) is transported across the mitochondrial membrane for final β-oxidation to generate acetyl CoA for the tricarboxylic acid (TCA) cycle. Both transport and oxidation are potential sites of regulation (Fig. 6).

It has been proposed that an increase in the mitochondrial enzymatic activity induced by exercise training is important for the increased ability of trained muscle to combust fatty acids during exercise (90). β-Hydroxy acyl CoA dehydrogenase (HAD) is a key enzyme in β-oxidation. Studies have shown that the activity of this enzyme is increased in conditions of increased fatty acid flux other than exercise such as a fat-rich diet (115). In agreement with a role of the enzymatic potential of the muscle in regulating fatty acid oxidation during exercise, a positive correlation between maximal LCFA uptake (r = 0.88, P < 0.05) or LCFA oxidation (r = 0.76, P < 0.05) and the activity of HAD was found across the working thigh (Fig. 7) (146). These findings suggest that the enzymatic capacity in the catabolic pathways is important in determining LCFA oxidation during exercise, although the exact molecular mechanism behind this relationship is not clear.

Regulation of LCFA-CoA flow into mitochondria results from the fact that acyl CoA derivatives cannot pass the mitochondrial inner membrane directly. First they have to be converted to their acyl carnitine derivatives. This reaction is catalyzed by the enzyme carnitine palmitoyltransferase 1 (CPT1) present at the outer mitochon-

![Fig. 6. Schematic representation of the likely routes taken for long-chain fatty acyl CoA (LCFA-CoA) to enter the mitochondrion for subsequent β-oxidation. CPT-1, carnitine palmitoyltransferase 1; CPT-2, carnitine palmitoyltransferase 2; ACS, acyl CoA synthetase; TCA, tricarboxylic acid cycle.](http://physrev.physiology.org/)

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The formation of malonyl CoA from acetyl CoA is catalyzed by the enzyme acetyl-CoA carboxylase (ACC), which exists in two isoforms (α- and β-form) of which ACCβ is dominating in skeletal muscle and heart (97) (Fig. 8). Two types of regulation of ACC have been described. The first is allosteric activation by the cytosolic concentration of citrate. In accordance, studies in rodents and humans revealed that high glucose availability at rest induced an elevation in cytosolic citrate concentration and in muscle malonyl CoA concentration (12, 224, 242, 243). The second type of regulation of ACC involves phosphorylation and inactivation by 5′-AMP-activated protein kinase (AMPK) (225, 242, 334).

AMPK activity is increased by exercise in human skeletal muscle. Thus an increase in the activity of α2- but not the α1-isoform of AMPK was demonstrated in human thigh muscle after bicycle exercise above 60% of maximal oxygen uptake but not after exercise of 50% of maximal oxygen uptake (82, 234, 284, 336, 338) unless the exercise was very prolonged (337). Moreover, during exercise, the AMPK activity is increased when muscle glycogen concentrations are low (234, 329, 336). During extremely intense sprint exercise, the α1-isoform is also activated (38). Accordingly, it would be expected that during exercise, activation of AMPK phosphorylates and inhibits ACCβ, thereby decreasing the concentrations of malonyl CoA. Another way to decrease the muscle content of malonyl CoA is by an increased activation of malonyl CoA decarboxylase (MCD), the enzyme responsible for decarboxylating malonyl CoA to acetyl CoA (Fig. 8). In accordance, it has been reported that electrical stimulation or exposure to the AMPK activator AICAR of rat skeletal muscle induced a two- to threefold increase in both MCD and AMPK activity (244). In contrast, another study showed that MCD is not a phosphorylation substrate of active AMPK and is not activated during muscle contractions in rat fast-twitch skeletal muscle (98). Thus, whether MCD is activated in muscle during exercise is at present not certain.

By lowering the malonyl CoA content in skeletal muscle, the inhibitory effect on CPT1 is diminished, and
the entry of acylated fatty acids into the mitochondria is facilitated. Relatively strong evidence has accumulated indicating that changes in ACC activity and malonyl CoA concentration regulate fatty acid entry into the mitochondria in resting muscle (reviewed in Ref. 195) and also during exercise in rodent muscle (225, 333, 334), but the question is whether the same regulatory mechanisms operate in human skeletal muscle during exercise. Earlier studies in human volunteers have failed to demonstrate decreases in malonyl CoA concentrations during prolonged submaximal exercise (202) and during short-term exercise at various submaximal intensities (203) despite marked increases in fatty acid oxidation compared with rest. However, recently we reported that during exercise at 60% of maximal oxygen uptake, malonyl CoA concentrations decreased compared with resting values, but the decrease was in fact similar when subjects were preconditioned with either low muscle glycogen levels and subsequently high AMPK activity or with high muscle glycogen levels and low AMPK activity (234), even though lipid oxidation was markedly different in the two conditions. These findings suggest that the decrease in malonyl CoA concentration from rest to exercise may contribute to the increase in free carnitine concentration when commencing exercise but plays a lesser role in fine tuning lipid oxidation during exercise. In another study exercise was performed with the knee-extensors at 60, 85, and 100% of the knee-extensor’s maximal oxygen uptake (56). Going from rest to 60% one-legged exercise, an ~50% reduction in ACC activity was associated with an increase in fatty acid oxidation, but no detectable change was noticed in the concentration of malonyl CoA. With increasing exercise intensities, at which the rate of fatty acid oxidation is diminished, the concentration of malonyl CoA was in fact modestly decreased and ACC activity was further reduced. From these human studies, it seems likely that other mechanisms than changes in ACC activity, AMPK activity, and malonyl CoA concentrations must be involved in the regulation of lipid utilization during exercise. In line with this suggestion are recent findings from studies performed in the perfused rat hindquarter in which low-intensity muscle contractions induced an increase in FA uptake and oxidation and a decrease in malonyl CoA muscle content without changes in total AMPK and ACC activities, suggesting that AMPK activation is not critical in the regulation of FA uptake and oxidation during low-intensity muscle contraction (223).

1. Regulation of fat oxidation by carnitine

Carnitine is substrate for CPT1 and is required for transport of the activated LC fatty acyl CoA across the inner mitochondrial membrane and is therefore essential for the β-oxidation (Fig. 6). Carnitine could thus play a role in regulating lipid oxidation. Available data from studies at rest suggest that fat oxidation in skeletal muscle is not limited by carnitine (221, 234, 275). Carnitine could, however, be a molecule that has important regulatory roles in adjusting lipid oxidation during exercise. The classical findings that carnitine can be acetylated by acetyl CoA (80) makes carnitine a sink for acetyl groups during conditions where the rate of acetyl CoA formation from pyruvate exceeds the rate of utilization by the TCA cycle. Accordingly, it has been demonstrated that the concentration of acetyl carnitine within the muscle is enhanced with increasing exercise intensities (49, 121, 203, 246, 312) decreasing the availability of free carnitine (49, 121, 246, 312), resulting in low CPT1 activity due to low availability of its substrate free carnitine. In turn, this will lead to a diminished supply of LCFA-CoA to β-oxidation, consequently limiting oxidation of fatty acids during exercise. This provides a potential mechanism whereby an increased availability of pyruvate and acetyl CoA formation can downregulate lipid oxidation (246). Supporting this view, an increase in muscle acetyl carnitine and decreased muscle free carnitine concentrations were observed concomitantly with a decrease in LCFA oxidation during increasing exercise intensity in male volunteers (312). Furthermore, we recently showed that when pre-exercise muscle glycogen concentrations were low, thereby providing few acetyl groups to acylate carnitine, muscle free carnitine concentrations and rate of fat oxidation were markedly higher during submaximal exercise than when preexercise muscle glycogen concentrations were high and free carnitine concentrations in skeletal muscle were low. The findings indicate that the availability of free carnitine may limit fat oxidation during exercise (234). Interestingly, when collecting data from various published studies where acetyl carnitine concentrations were measured in skeletal muscle and substrate utilization was estimated from RER values during exercise, a close positive relationship between acetyl carnitine concentrations and RER values and a negative relationship between acetyl carnitine and fat oxidation is found (Fig. 9). Because total carnitine concentration in skeletal muscle is unaffected by exercise, this relationship suggests that high RER values and low fat oxidation may be related to the availability of free carnitine in human skeletal muscle during exercise. Putting it differently the positive relationship in Figure 9 suggests a relationship between free carnitine concentration in contracting muscle and muscle fat oxidation rate. Even though CPT1 no doubt is the central gatekeeper for entry of fatty acyl moieties into the mitochondria, it was recently shown in isolated mitochondria from rat muscle that FAT/CD36 is located in the mitochondrial membrane and immunoprecipitation with FAT/CD36 antibody coprecipitates CPT1 (33). This is interesting but surprising since morphological studies do not reveal such localization (143, 320). It was
suggested that FAT/CD36 could play a role in LCFA transfer across the mitochondria membrane likely in combination with CPT1 (33), but further work is needed to investigate this possibility.

Integrating the various findings in muscle during exercise leads to a schematic presentation of the possible roles of AMPK, ACC, and carnitine in the regulation of lipid oxidation during exercise (Fig. 10).

2. Other potential regulators of fat oxidation

Other factors involved in the regulation of LCFA utilization during exercise could be considered. It has been shown in vitro in human skeletal muscle that a decline in pH from 7.1 to 6.8 resulted in a significant decrease of 34–40% in CPT1 activity both in sarcolemmal and interfibrillarily located mitochondria (16, 280). A link between pH and CPT1 makes sense, as during prolonged submaximal exercise when lipid utilization is high, only a small increase in muscle lactate and a small decrease in muscle pH is observed in contrast to exercise performed at high intensities, where high levels of muscle lactate have been obtained in parallel with reductions in pH (245) and lipid oxidation. Thus an exercise-induced decrease in pH causing a decrease in CPT1 activity could also seem to be a likely mechanism contributing to a decrease in lipid oxidation during high-intensity exercise.

Another step to consider is the \( \beta \)-ketoacyl-CoA thiolase, the enzyme responsible for catalyzing the final reaction in \( \beta \)-oxidation: \( \beta \)-ketoacyl CoA to acetyl CoA and acyl CoA (Fig. 6). \( \beta \)-Oxidation is inhibited mainly by feedback inhibition and acetyl CoA has been shown to be a potent inhibitor of \( \beta \)-ketoacyl CoA thiolase. Therefore, when acetyl CoA is accumulating the thiolase is inhibited and accordingly so is \( \beta \)-oxidation. In fact, inactivation of the thiolase causes the complete inhibition of palmitate \( \beta \)-oxidation (177). Thus high concentrations of acetyl CoA, as observed during intense exercise in muscle (49, 62, 203) or when muscle glycogen levels are high (221, 234), may tend to slow \( \beta \)-oxidation by inhibition of \( \beta \)-keto acyl CoA thiolase.

Finally, changes in the membrane lipid composition may affect the kinetic properties of different membrane-associated enzymes and transporters, which could influence the LCFA uptake. Recently it was demonstrated that exercise training and diet induce modifications of the phospholipid composition of the membranes in human skeletal muscle (7, 8, 118). The functional role of a change in membrane phospholipid composition on transsarcolemmal transport of LCFA should be elucidated.

IV. CIRCULATING VERY-LOW-DENSITY LIPOPROTEIN-TRIACYLGLYCEROL

Endogenous triacylglycerol (TG) is secreted by the liver, wrapped up into VLDL. VLDLs are the main carriers of circulating TG in the postabsorptive state. The hydrolysis of core triacylglycerol in VLDLs is mediated by lipoprotein lipase.

Most authors have neglected the potential contribution of fatty acids derived from circulating VLDL-TG to energy substrates during exercise. This might be due to earlier findings where the arteriovenous (a-v) differences of total serum triacylglycerols (S-TG), both unlabeled and radiolabeled, were measured in subjects during forearm exercise (204). The authors concluded that a-v differences of S-TG did not exceed the level of error of the analytical methods used in the study. On the other hand, they also estimated that due to the accuracy of the methods, a-v differences of up to 20 \( \mu \)M could have been missed and such an a-v difference could, if fully oxidized, account for ~25% of the oxygen extraction in the forearm due to the energy density of circulating TG. Thus the potential for a
significant contribution of circulating S-TG was not ruled out. In addition, findings in the forearm may not be completely representative to findings in the leg. For example, Havel et al. (109) demonstrated a consistent 4–6% arterial-femoral venous difference of circulating triacylglycerol during exercise in two out of four subjects, whereas in the other two subjects the differences were small at 1%. From these data it was concluded that the circulating triacylglycerols could contribute 10% of the necessary fuels during exercise. However, in evaluating the possible contribution of circulating triacylglycerol, it is necessary to consider that because TG is so energy-dense a significant contribution from circulating VLDL-TG to the oxidative metabolism requires only a very small fractional extraction. Furthermore, because VLDL-TG is the main carrier of TG in the overnight fasted state, it may be more appropriate to measure VLDL-TG than total serum TG. Thus, when TG in VLDL was measured in the femoral artery and vein at rest and during exercise, a consistently lower VLDL-TG concentration was observed in the femoral vein than in the artery under resting conditions, and this VLDL-TG degradation was significantly larger when measured in a trained leg compared with a nontrained control leg (150). Although consistent femoral a-v differences of VLDL-TG were not found at all time points in all subjects during submaximal exercise with the knee-extensors, the total net degradation of VLDL-TG during 2 h of exercise, estimated from the area under the curve of femoral arterial and venous VLDL-TG multiplied by plasma flow, averaged 8 mmol in the nontrained leg and was a little higher in the trained leg (9.3 mmol) (147). The finding of a degradation of VLDL-TG during exercise was supported in a recent study, where submaximal bicycle exercise was performed for 1 h. In that study male volunteers had been on a fat-rich diet [65 energy (E%) fat] for 7 wk while participating in an endurance training program. After 30 min of exercise, the average femoral a-v difference of VLDL-TG amounted to ~100 μM, and the estimated energy delivered by VLDL-TG during the hour of exercise covered ~25% of total energy fuels and ~40% of lipid fuels, assuming that all liberated fatty acids were oxidized (117). When comparable subjects ingested a carbohydrate-rich diet (65 E%) during 7 wk of training, the degradation of serum VLDL-TG across the leg during ex-
ercise was significantly less in spite of higher serum VLDL-TG concentrations (117) and contributed only ~5% of total energy fuels and ~10% of lipid fuels. Supporting a role for serum VLDL-TG as a fuel during exercise, an increase compared with rest in VLDL turnover was observed during moderate-intensity exercise in human volunteers even though plasma VLDL-TG concentrations remained constant during the exercise period (198). Taken together, the studies suggest that circulating VLDL-TG may contribute to energy delivery to human skeletal muscle during exercise. The magnitude of this contribution may vary depending on the habitual diet of the subjects, exercise intensity, and duration and may reach 25% of total energy expenditure after adaptation to a fat-rich diet. Because of the energy-dense nature of VLDL-TG, only a very small fractional extraction in muscle is sufficient to cover a substantial amount of energy provision during exercise. Clearly more research is needed to clarify the potential role of serum VLDL-TG as a fuel in various exercise modes and durations of exercise. It is worth considering that neglecting the contribution of serum VLDL-TG to fuel provision during exercise will cause an overestimation of IMTG utilization during exercise. The magnitude of this contribution may vary depending on the habitual diet of the subjects, exercise intensity, and duration and may reach 25% of total energy expenditure after adaptation to a fat-rich diet.

Because of the energy-dense nature of VLDL-TG, only a very small fractional extraction in muscle is sufficient to cover a substantial amount of energy provision during exercise. Clearly more research is needed to clarify the potential role of serum VLDL-TG as a fuel in various exercise modes and durations of exercise. It is worth considering that neglecting the contribution of serum VLDL-TG to fuel provision during exercise will cause an overestimation of IMTG utilization during exercise in studies where IMTG utilization is estimated as the difference between total lipid utilization determined by indirect calorimetry and whole body isotope-derived LCFA utilization (LCFA $R_d$), as described in section $vB$.

A. Skeletal Muscle Lipoprotein Lipase

As discussed above, it is apparently not the arterial VLDL-TG concentration that is determining the degradation of VLDL-TG in exercising muscle (117, 198) but rather local factors in the muscle, of which the lipoprotein lipase (LPL) activity is important. The activity of LPL might, on the other hand, also be influenced by the oxidative potential of the skeletal muscle as will be discussed later.

Since VLDL-TG is degraded by LPL, it is appropriate to consider whether the activity of muscle LPL is increased by exercise, since this might facilitate breakdown of VLDL-TG during exercise. LPL is located at the luminal site of the endothelial cells in the capillary bed of primarily skeletal and cardiac muscle and adipose tissue. LPL is the rate-limiting enzyme in VLDL-TG hydrolysis, and the action of LPL makes the liberated fatty acids available for uptake in the surrounding tissue. LPL is thought to primarily hydrolyze circulating lipoproteins, while it is associated with heparan sulfate proteoglycans (HSPG) to the capillary endothelium. After synthesis in the myocyte, the enzymes must transfer to the endothelial cells and then translocate from the abluminal to the luminal surface of the capillary endothelial cell. Final posttranslational modifications of LPL involve dimerization and asparagine-linked glycosylation in the endoplasmic reticulum and Golgi complex (89, 220) to become an enzymatically active enzyme.

To evaluate whether exercise and exercise training induce an increase in skeletal muscle LPL activity, it is important to control for the nutritional status, as this has a significant influence on muscle LPL activity (148, 290). When dietary control was performed, a 70% higher muscle LPL activity in the vastus lateralis muscle was demonstrated after 8 wk of regular, dynamic knee-extension exercise training compared with the untrained muscle in the same individuals (150), indicating that training by itself increases muscle LPL activity. This finding was further supported in a recent cross-sectional study showing that males and females, who had been engaged in regular physical exercise training for several years, had significantly higher muscle LPL activity than sedentary matched males and females (155). It is well known that endurance training increases the capillarization of skeletal muscle (6, 150, 249). This augmented capillarization will increase the surface of the endothelium, which offers the possibility to anchor more muscle LPL to the binding sites at the luminal endothelial cell surface. Accordingly, a close correlation between capillarization and muscle LPL activity has been demonstrated (150). An increased capillarization also results in an improved perfusion of the tissue, decreased diffusion distances, and decreased mean transit time (248). The latter may increase the possibility of a longer contact time between the VLDLs and LPL, which in turn may increase lipolysis. Moreover, the capacity to oxidize lipids is enhanced in the trained state because of the training-induced increases in the activity of oxidative enzymes (120, 124, 147, 249). Data have suggested that when the capacity of tissue to metabolize the liberated fatty acids is exceeded, hydrolysis slows down, primarily as a result of product inhibition of LPL (212). Thus an increased capacity for muscle lipid oxidation may increase the assimilation of fatty acids and result in less product inhibition of LPL.

Different findings exist as to whether acute exercise induces changes in muscle LPL activity. In some studies where one-legged knee-extension exercise was performed for 1–2 h (150, 151) or bicycle exercise for 1 h (178), muscle LPL activity, measured in biopsies obtained at termination of exercise, remained unchanged compared with rest. This is in contrast to findings demonstrating a significant increase in muscle LPL activity at termination of exhaustive prolonged bicycle exercise in healthy male volunteers (153) (Fig. 11). If total energy expenditure during exercise plays a role for the increase in muscle LPL activity, that might be the reason for the discrepant findings. Alternatively, exercise-induced increases in muscle LPL activity may take time to occur. Accordingly, under conditions where muscle LPL activity was not enhanced at termination of exercise, a 4-h de-
A delayed increase in the enzymatic activity was observed (Fig. 12) (151), and the increase in muscle LPL activity has in some situations lasted from several hours up to at least 24 h (Fig. 11) (153, 179, 264).

Recent investigations have shed some light on the regulatory mechanisms underlying the cellular LPL response to exercise in muscle. Hence, muscle LPL mRNA levels are not changed immediately after 90 min of submaximal exercise in male or female subjects (155), but another study revealed an exercise-induced delayed increase (4 h postexercise) in muscle LPL mRNA expression (265) followed by an increase in muscle LPL protein 8 h postexercise (265). These data are supported by the findings of no elevation in transcription rate of LPL in the vastus lateralis muscle immediately after a 4-h low-intensity cycling bout or after a 60- to 90-min one-legged knee-extensor exercise (216). However, the transcription rate increased steadily to three- to sixfold above control levels after 4 h of recovery. LPL mRNA expression tended to increase in both conditions but was not statistically significant (216). Thus it is tempting to speculate that the metabolic demands, induced during exercise, lead to an activation of the LPL gene and LPL activity in skeletal muscle to ensure sufficient energy for the muscle during recovery but also that even small, transient increases in the transcription rate of muscle LPL generate a cumulative increase in mRNA and subsequently in the protein content after regular exercise training for several weeks or months. Accordingly, not only muscle LPL activity but also muscle LPL mRNA expression was higher in trained than sedentary males and females (148, 155, 290).

A variety of mechanisms may regulate LPL activity in skeletal muscle in the postexercise period. During an euglycemic hyperinsulinemic clamp, we demonstrated a decrease in resting muscle LPL activity at physiological insulin concentrations (151), which is in contrast to the stimulatory effect of insulin on LPL in adipose tissue (Fig. 12) (206). In contrast to these findings, an enhanced muscle LPL activity for at least 24 h postexercise was demonstrated in spite of the ingestion of a carbohydrate-rich diet (65–70 E%), resulting in enhanced insulin concentrations and high muscle glycogen resynthesis rate (153) (Fig. 11). Others have also demonstrated a prolonged increase in muscle LPL activity despite intake of food (179, 264). These observations may then indicate that prior exercise makes muscle LPL activity resistant to the inhibitory effects of insulin. In agreement with this interpretation, we previously demonstrated that whereas insulin decreases muscle LPL activity in resting muscle, the effect of insulin is apparently counterbalanced by other mechanisms in muscle after exercise (151) (Fig. 12). One such "exercise" factor could be a low muscle glycogen content. However, the increase in muscle LPL activity during recovery is apparently not directly related to changes in muscle glycogen concentrations, since we found that muscle glycogen was decreased at termination of an exercise bout in parallel with unchanged muscle

![FIG. 11. Intramyocellular triacylglycerol (IMTG) content and muscle lipoprotein lipase (mLPL) activity in the vastus lateralis muscle obtained at rest, immediately after exhaustive prolonged glycogen-depleting exercise, and in the postexercise recovery period. *P < 0.05 compared with preexercise values of IMTG. †P < 0.05 compared with preexercise values of mLPL activity. [Data from Kiens and Richter (153).]

![FIG. 12. Muscle lipoprotein lipase activity in the nonexercised (●) and exercised (○) thigh in the control (top) and clamp (bottom) groups. In the clamp group, the steady-state insulin concentrations were 23 and 44 μU/ml at 6 and 8 h of recovery, respectively. In the control group, the lipoprotein lipase activity was measured before (pre) and after (post) one-legged knee extensions as well as during recovery for 4 and 8 h. *P < 0.05 compared with contralateral thigh. †P < 0.05 compared with previous measurement. [From Kiens et al. (151), copyright 2000, The Endocrine Society.]
LPL activity (151). Furthermore, during fasting recovery, muscle glycogen remained unchanged, whereas LPL activity increased after 4 h and then decreased towards preexercise levels after 8 h of recovery (151) (Fig. 12) still in the face of unchanged low muscle glycogen levels. Interestingly, during the postexercise recovery period, the maximum activity of muscle LPL activity was observed at the same time as the muscle TG was decreased the most, which was 18 h after termination of exercise (153) (Fig. 11). As muscle LPL activity is responsible for VLDL-TG hydrolysis, it was suggested that the fatty acids liberated from VLDL-TG hydrolysis might be a potential energy source during postexercise recovery (153). In accordance with this hypothesis, Malkova et al. (191) reported a significant clearance of total triacylglycerol and VLDL-TG across the leg during a postprandial period 14 h after leg exercise. Similarly, in six healthy male volunteers studied during 6 h of recovery from intense glycogen-depleting exercise with one leg, the degradation of VLDL-TG across the previously exercised leg (4.8 ± 1.0 mmol) was marked edly higher than over the nonexercised control leg (1.8 ± 0.6 mmol) (Kiens, unpublished observations), and furthermore, VLDL-TG fractional catabolic rate was significantly higher during the early recovery phase than at rest and during exercise in human volunteers (198).

Collectively the studies indicate that muscle LPL activity is unchanged during moderate exercise and increased when exercise is strenuous and prolonged, hence allowing hydrolysis of circulating VLDL-TG to occur. On the other hand, in the postexercise recovery period after exhaustive and glycogen-depleting exercise, there seems to be a marked degradation of VLDL-TG across the legs in fed subjects despite enhanced plasma insulin levels. This seems to be caused by a marked increase in muscle LPL activity, transcription, and translation induced by exercise. Thus it is tempting to speculate that the metabolic demands, induced during and/or after exercise, lead to an activation of the LPL gene in skeletal muscle to secure lipids as energy fuel while the resynthesis of glycogen is of priority.

V. INTRAMYOCYTOULAR TRIACYLGLYCEROL

Triacylglycerol stored within striated muscle cells (intramyocellular triacylglycerol, IMTG) represents a potentially large energy source, and several factors influence the IMTG content in skeletal muscle as discussed below.

A. Factors Affecting Intramyocellular Triacylglycerol Content

1. Diet

One of the factors determining the content of TG within human skeletal muscle is the composition of the diet. When healthy, physically active male volunteers increased their fat intake to 54 E% for 4 wk, a significant increase in IMTG content of 50% was observed (148). Similarly, 7 wk of endurance training of healthy male subjects consuming a fat-rich diet (65 E%) increased IMTG content by 57%. In contrast, when similar subjects were subjected to a high-carbohydrate diet (85 E% carbohydrates) for 7 wk of training, IMTG content remained unchanged compared with the pretraining level obtained on the habitual mixed diet containing 30 E% of fat (117). Along this line, Coyle et al. (54) demonstrated that a decrease in dietary fat intake to 2 E% for 3 wk resulted in a decrease in IMTG content. Other reports also indicate that IMTG content is dependent on the fat content of the habitual diet and in the postexercise recovery period (119, 279, 342). Collectively, these data clearly demonstrate that a fat-rich diet increases and a carbohydrate-rich diet decreases the triacylglycerol content in skeletal muscle.

The triacylglycerol pool within the muscle receives LCFAs from circulating albumin-bound fatty acids and from VLDL-TGs that are degraded by LPL. Thus the dietary influence on IMTG concentrations may be related to the activity of muscle LPL. The consequence of an increased amount of fat in the diet is an increased activity of LPL in skeletal muscle along with increased IMTG (148). On the other hand, a diet rich in carbohydrates for 3 days (139) or 4 wk after the consumption of a diet rich in fat (148) both reduced LPL activity in skeletal muscle. The reciprocal variation between dietary carbohydrate amount and LPL activity could possibly be explained by the generally higher plasma insulin levels when fed a carbohydrate-rich diet because insulin is found to decrease LPL activity in skeletal muscle (Fig. 12) (151).

Thus these findings support the notion that diet-induced changes in muscle LPL activity play a significant role in the dietary-induced changes in IMTG. The likelihood of this possible association is further strengthened by the fact that when training is performed while ingesting a fat-rich diet (65 E%) the increase in IMTG correlates tightly with the increase in muscle LPL activity (Fig. 13).

2. Muscle fiber type

While the above-described studies make a strong case for the diet as an important determinant for IMTG content in resting skeletal muscle, other aspects are of importance too. For example, as alluded to earlier, concentrations of IMTG are muscle fiber type specific. Previous biochemical analysis on single fibers and histochemical and electron microscopy studies have shown up to a threefold higher lipid content in type 1 fibers than in type 2 fibers in healthy male subjects (66, 130) and in rats (277). These data are supported by recent data using oil red O and immunofluorescence microscopy (316, 317). Data obtained by 1H-magnetic resonance spectroscopy
(\(^1\)H-MRS) in skeletal muscle also suggest a fiber type difference in IMTG. Thus, when the lipid content was quantified in the soleus and tibialis anterior muscle in humans, a two- to threefold higher intramyocellular TG content was found in the soleus muscle relative to that in tibialis anterior (134, 210, 230). That the amount of triacylglycerol in a muscle is dependent on the fiber type distribution within the muscle was supported by our finding in the vastus lateralis muscle of a positive correlation between the percentage of type 1 fibers in the quadriceps muscle expressed relative to fiber area on one hand and the IMTG content on the other hand (\(r = 0.39, n = 41, P < 0.05\)) (281).

3. Gender

Gender may also play a significant role in triacylglycerol content of skeletal muscle. Recently we demonstrated that females have a higher resting IMTG concentration than male subjects independent of training status (281). The gender differences could not be ascribed to the influence of the diet as both female and male subjects had consumed a similar diet during the preceding 8 days. Whether the phase of the menstrual cycle plays a role for the content of lipids in skeletal muscle is not clear. However, in the above-mentioned study, all females were studied in the midfollicular phase of the menstrual cycle. As mentioned above, previous studies have shown up to a threefold higher lipid content in type 1 fibers than in type 2 fibers in healthy male subjects (66, 130, 179, 316, 317). Whether this pattern also exists in females or whether both type 1 and/or type 2 fibers in females contain an increased amount of lipids compared with males remains to be elucidated. Gender differences in lipid content in regard to different fiber types by using the \(^1\)H-MRS method are not clear due to the limited number of subjects included in the studies (134, 210).

4. Physical training

Longitudinal training studies have shown higher concentrations of IMTG in the vastus lateralis muscle in healthy subjects after endurance training for 2 wk (261), 6 wk (130), or 31 days (214). Also when exercise training was allocated to a single muscle group (the knee-extensors) in subjects on a mixed diet for 8 wk, a higher IMTG content was apparent compared with IMTG content in the contralateral, untrained leg (147). This indicates that training by itself may have an enhancing effect on IMTG levels. Recently, IMTG content, measured in vastus lateralis muscle 48 h after an exercise bout by using the oil red O soluble dye, was found to be significantly higher in trained versus untrained, lean male subjects (91), and similar findings have been made with the MRS technique (58). In contrast, when female and male volunteers at different physical activity levels were studied with the muscle biopsy technique, IMTG content in the vastus lateralis muscle was found to be independent of training status (281). The lack of training effect on IMTG content might very well be caused by the fact that in the latter study all subjects followed a similar, controlled carbohydrate-rich diet during the preceding 8 days. Because the IMTG content is strongly dependent on the diet (as discussed above), it is suggested that a training-induced enhancement of IMTG content in males and females is dependent on the diet consumed such that ample amounts of fat in the diet are necessary for an increase in IMTG with training to occur.

B. Methodological Considerations

Essentially two different methods have been utilized to measure muscle triacylglycerol content. These are the muscle biopsy technique with subsequent biochemical or microscopic determination of IMTG content and the magnetic resonance spectroscopy technique \(^1\)H-MRS. For both techniques a methodological problem is to distinguish between intracellular and intercellular TG. The latter is located in adipocytes associated with the muscle cells, whereas the former is found in the form of lipid droplets inside the myocytes.

I. Biochemical determination

The classical method to determine muscle triacylglycerol has been to obtain a piece (5–10 mg wet wt) of muscle by biopsy and then to freeze-dry it. Subsequently, the piece of muscle is dissected free of blood, connective tissue, and adhering adipose tissue under a microscope,
leaving the individual muscle fibers for analysis. The technique has been criticized for having a large coefficient of variation as described by Wendling et al. (332). This has been thought to be due to invisible contamination of the muscle fibers with adipocytes or remnants of adipocytes. Because the triacylglycerol content in adipocytes is large compared with the content inside the fibers, contamination with a few adipocytes would greatly influence the measurements of TG in muscle tissue. Still, it is apparent from personal experience and from experience from colleagues in the field (315) that after examining many muscle biopsies as well as histochemical sections from healthy young human subjects, the presence of adipocytes interspersed between muscle fibers is very infrequent. To further elucidate whether contamination of dissected muscle fibers with adipocytes is an issue, we have performed Western blotting of dissected muscle and of adipose tissue and have blotted for perilipin, a protein which is abundant in adipocytes (Fig. 14). As can be seen from the blot in Figure 14, while a strong signal as expected is found in adipocytes, no signal is found in muscle loaded at considerably higher protein content, indicating no or extremely little contamination with adipocytes (Fig. 14A). In agreement with our findings, it was previously reported that the mRNA expression of adipsin, which is expressed at a high level in adipocytes, was undetectable in carefully dissected skeletal muscle samples, indicating no contamination of the muscle with adipose tissue (171) (Fig. 14B).

A putative explanation for the variability in biochemical determination of IMTG in a muscle biopsy could be that different biopsies contain different percentages of type 1 and type 2 muscle fibers, and because the IMTG content is two to three times higher in type 1 fibers than in type 2 fibers (66, 130, 316), this could introduce some variability in the results, especially when muscles with mixed fiber type distribution are studied. It is, however, also worth considering that a muscle biopsy obtained during exercise is a mixture of active and nonactive or less active fibers, especially during low-intensity exercise. As reesterification of LCFA into IMTG can take place in nonexercising muscle (262) and probably also in nonactive fibers, results from a biopsy of mixed muscle will reflect these different responses and different fiber types, in turn increasing variation in the results.

To minimize variation due to fiber type differences between biopsies, one can examine a larger sample (30–40 mg wet wt) that is freeze-dried and dissected as described above into single fibers, which are then mixed before the biochemical analysis (152). The advantage of this method is that an initial large muscle sample from which pooled single fibers are picked will better represent

FIG. 14. A: Western blots of perilipin in human adipose tissue (AT) and the vastus lateralis muscle in female (F) and male (M) volunteers. Note that muscle was loaded at 40 μg protein, whereas adipose tissue was loaded at 3 μg protein. B: mRNA for various lipid binding proteins and for the adipocyte specific protein adipsin in carefully dissected human muscle biopsies. Note absence of adipsin mRNA in muscle samples. [From Lapsys et al. (171).]
the muscle. With the use of this approach, a much smaller coefficient of variation (CV) of the IMTG concentration of 4% has been obtained between five samples from the mixed freeze-dried pool of fibers as described above. This procedure is in contrast to the situation when a muscle sample of 40 mg is divided into five small 5–10 mg samples of wet muscle that are subsequently dissected and analyzed separately resulting in a CV of 31% (281) similar to the value obtained by Wendling et al. (332).

In several laboratories the Folch method is used for extraction of lipids from the tissue. Exchanging the laborious and repeated extractions by the Folch method (where recovery is not 100%) with incubation with tetraethylammonium hydroxide, which selectively cleaves triacylglycerol in tissues (152), also helps reduce variability in the assay. Performed carefully, the muscle biopsy technique remains the only definitive method for quantification of myocellular triacylglycerol.

2. Morphological determination

Electron microscopy has also been used to examine sections from muscle biopsies. Whereas this technique offers unprecedented detailed information, the large magnification makes it difficult to examine a large number of muscle cells (128). Staining of muscle sections with oil red O and subsequent analysis by light microscopy has also been used to estimate muscle IMTG content (91, 190). Recently this technique has been combined with fluorescence microscopy, which enables direct visualization of both intramyocellular triacylglycerol (IMCL) deposits and muscle fiber type in the same muscle cross section (57, 316, 317). Applying this method for analyzing TG content in muscle supported the earlier findings of fiber type specific IMTG content obtained by biochemical analysis of single muscle fibers (66). Because the histochemical technique is based on light microscopy, it allows a large number of fibers to be analyzed from the same biopsy. However, it should be noted that visualization of IMTG droplets either by electron microscopy or by light microscopy only allows a semi-quantitative measure of IMTG.

3. $^1$H-MRS

With the $^1$H-MRS technique, the signal from IMCL can be at least partially separated from the signal from extramyocellular triacylglycerol (EMCL) because the spectra are slightly different (21). The advantage of the method is obviously the noninvasive nature of the technique that allows unlimited measurements and apparently a separation between IMCL and EMCL. However, the $^1$H-MRS technique contains a number of limitations and disadvantages. These include the difficulties in positioning the voxel to avoid vasculature, minimizing inclusion of subcutaneous adipose depots, and ensuring consistent orientation of the muscle fibers along the magnetic field. In addition, the presence of EMCL can cross-contaminate the IMCL signal whereby the precision of the IMCL estimation will be reduced. Another disadvantage is that the scans take at least 15 min (20), which in combination with the time it takes to carefully position the voxel often causes IMTG determinations to be performed 1–2 h after termination of exercise and opens the possibility that measurements reflect postexercise triacylglycerol levels rather than levels immediately after exercise. Furthermore, until recently, most $^1$H-MRS studies have been performed on the soleus or the tibialis anterior muscles, whereas most biopsy studies are performed on the vastus lateralis muscle making comparisons difficult. However, recently $^1$H-MRS studies of the vastus lateralis muscle have also been published, and the findings from these studies basically confirm findings in the earlier studies on the soleus and tibialis anterior muscles (142, 262). A validation of the $^1$H-MRS studies has been done in dogs, where the tissue levels were measured at rest without prior interventions such as exercise, and the accuracy of the method was comparable to the biochemical determination (291). In humans, results obtained before and after exercise showed a good agreement between $^1$H-MRS data and electron microscopy of biopsies obtained from the tibialis anterior muscle, whereas the biochemical extraction method gave different results (131).

4. Indirect measurements

In addition to these methods for measuring IMTG content in muscle, a third method has been used extensively to estimate utilization of IMTG during exercise. This is the indirect method based on the difference between whole body lipid oxidation, calculated by indirect calorimetry, and the plasma rate of disappearance ($R_d$ FA) or oxidation of fatty acids calculated from tracer techniques. As discussed in section vC3, this method has significant limitations.

C. Intramyocellular Triacylglycerol Utilization During Exercise

For many years it has been debated whether triacylglycerols located in the muscle are utilized during exercise because conflicting results have appeared. With a closer look at the experimental designs of the different studies, it is striking that the exercise tests performed have varied in intensity and duration from minutes to several hours, and the training status of the subjects has varied from untrained to trained. Moreover, different exercise models have been employed, and finally the method by which IMTG was measured differs among studies. To interpret the role of IMTG as a potential
energy source during exercise, these matters have to be taken into consideration.

1. Studies where the muscle biopsy technique was applied

In studies where the muscle biopsy technique was used in males by Carlson et al. (34) and Costill et al. (53), a decline of 25% in IMTG was obtained after 99 min of bicycle exercise and 30% after 147 min of exercise on a treadmill, respectively. In both of these studies IMTG was measured on a wet muscle sample from a biopsy, where dissection of tissues and material other than muscle (such as connective tissue, blood, and adipocytes) is difficult. Using a similar procedure of analysis of IMTG, Fröberg and Mossfeldt (81) and Lithell et al. (179) found a decrease of 50% of IMTG during exercise. However, in those studies the subjects exercised for 7–9 h (the classical Swedish Wasa ski-race), and this large energy demand is expected to recruit all energy sources heavily. Interestingly, the most fit skiers had the largest stores of IMTG before the race, and they also depleted the stores the most (179). Findings of an exercise-induced decrease in muscle triacylglycerol content in males have also been reported in later studies, where freeze-dried and dissected muscle tissue has been used for the analysis of IMTG and exercise was performed for −2 h (64, 133, 214), whereas other studies did not find detectable changes in IMTG during exercise (14, 117, 147, 153, 235, 279, 281). Lack of breakdown of IMTG was also shown indirectly by microdialysis of the vastus lateralis muscle during exercise. During dynamic knee-extensions at 65 and 85% of knee-extensor peak oxygen uptake in male volunteers, an interstitial water-arterial plasma water gradient of glycerol was not observed, indicating a lack of breakdown of muscle triacylglycerol (278). A muscle biopsy at termination of exercise confirmed this finding. It is, however, noteworthy that females of widely differing training backgrounds in contrast to matched male volunteers utilize a significant amount of IMTG in the vastus lateralis during prolonged bicycle exercise (281). The significant utilization of IMTG in females could be caused by the higher habitual IMTG levels in females than in males and/or that type 1 muscle fibers expressed per area were more abundant in females than in males (281).

When combining the muscle biopsy technique with the oil red O staining, it was found in males that prolonged submaximal exercise for 2 h resulted in significant reduction in area, of IMTG percent, but only in type 1 fibers (57, 317). Interestingly, IMTG breakdown during exercise occurred only in the fasted state and was completely abolished when subjects were fed carbohydrate before and during exercise (57).

2. Studies where the 1H-MRS technique was applied

When treadmill running at 65–70% \( V_{O_2\text{peak}} \) was performed until exhaustion or for 2 h, a decrease by 33% in the IMTG signal (IMCL in MRS terminology) content in soleus muscle was obtained in seven males and two females (167) and by 25% in females (172). Treadmill walking at 50% \( V_{O_2\text{peak}} \) for 2 h decreased IMCL signal in the tibialis anterior muscle of males by 22–26% (58), whereas running at alternating intensities for 90 min or to exhaustion did not decrease the IMCL signal in soleus, gastrocnemius, or tibialis muscles of males (230). In another study, utilization of IMCL in males was found to be dependent on the intensity of the exercise because running at 60–70% of maximal oxygen uptake decreased IMCL signal in both tibialis anterior and soleus muscles, whereas running at 80–90% of maximal oxygen uptake did not cause changes in IMCL signal in either muscle (27).

Thus the above 1H-MRS studies suggest breakdown of IMTG during prolonged low- to moderate-intensity exercise, whereas during intermittent or high-intensity exercise, no detectable breakdown occurs. In agreement with this interpretation, recent 1H-MRS measurements performed on the vastus lateralis muscle reported a 20% decrease in IMCL signal in trained male cyclists after 3 h of exercise at 55% of maximal power output (262). Likewise, when strenuous exercise was performed at 70% \( V_{O_2\text{max}} \) for 3 h in highly trained male cyclists, after the consumption of either a high- or a low-carbohydrate diet (48 h), a reduction in IMCL signal of 57 and 64%, respectively, was obtained (142). However, the measurements were not performed until 1–2 h after termination of exercise. Breakdown of IMTG in the early recovery phase has been found in some (57, 153) but not in another study (160), and therefore, it cannot be excluded that a delay of 1–2 h in obtaining postexercise IMTG concentrations may influence the data obtained.

3. Indirect estimation of myocellular triacylglycerol utilization during exercise

With the use of the indirect method of quantification of IMTG utilization during exercise and with the assumption of 100% oxidation of \( R_d \) of plasma LCFA, it has been estimated that nonplasma sources assumed to be intramuscular TG provide 35–50% of total lipid oxidation in male volunteers during exercise at various durations and intensities (55, 140, 193, 215, 239). This indirect measure is, however, based on several assumptions. For instance, if part of the circulating fatty acids is stored rather than oxidized, then the apparent contribution of IMTG to fat oxidation is underestimated. As discussed previously, the percentage of LCFA-\( R_d \) oxidized varies with exercise intensity. Finally, the greatest limitation in the use of the indirect estimation of triacylglycerol breakdown is the finding that only 34–60% of whole body LCFA disappear-
ance ($R_d$) during bicycle exercise can be accounted for by uptake in the working legs, as discussed in section ii. This means that a major part of $R_d$ for LCFA during exercise in fact occurs in other parts of the body than the exercising legs. Furthermore, it is assumed that LCFA is the only circulating lipid source, thereby ignoring VLDL-TG as a potential energy source. As discussed in section iv, this may not be correct. It therefore seems fair to conclude that this indirect method is inadequate to estimate utilization of intramuscular triacylglycerol during exercise, and therefore, studies using this method for quantification of IMTG are not easy to interpret. However, in a recent study the tracer technique was used combined with histochemical analysis of muscle. During 120 min of exercise at 60% of $V_O2_{max}$ in trained male cyclists, it was shown that muscle- and lipoprotein-derived TG measured by the tracer technique covered ~15% of total energy expenditure. At the same time, a net decline in muscle lipid content was demonstrated in the type I fibers only, quantitated by the oil red O analysis (314). Unfortunately, probably due to the semi-quantitative nature of the oil red O determination of IMTG utilization, a direct comparison of the two methods for quantification of IMTG utilization during exercise was not reported by the authors. The findings in that study also revealed that the rate of LCFA oxidation increased substantially during exercise concomitantly with a decline in the rate of muscle- and lipoprotein-derived TG oxidation (314). These observations support earlier suggestions by Kiens et al. (147) that enhanced utilization of blood-borne fat substrates could decrease and/or inhibit intramuscular TG net breakdown.

D. Triacylglycerol Stored in Adipocytes Associated With Muscle

A potential source of lipids during exercise might be fatty acids liberated from adipocytes dispersed between or along the muscle fibers as suggested previously by us and others (65, 117, 147, 235). From muscle biopsies it is not possible to get a good measure of the intercellular TG pool. However, because the in vivo $^1$H-MRS studies apparently can differentiate between the IMCL and the EMCL signal, a possibility may exist to evaluate whether breakdown of a triacylglycerol compartment, located in between the muscle fibers, takes place during exercise. In a recent study in which IMCL signal decreased during exercise in female runners (172) and in trained male cyclists (262), no significant change in EMCL signal was observed. In another $^1$H-MRS study in which no change in IMCL with prolonged intermittent exercise was found, no changes in EMCL were found either (230). However, the variation in measurements of EMCL apparently is considerable (172, 291), and in most $^1$H-MRS studies on exercise, EMCL values are not reported. In fact, Decombaz et al. (58) state that “because the size of the EMCL signal in a specific spectrum is not representative for a specific muscle, the size of the EMCL signal cannot be evaluated in terms of depletion and recovery.” If so, the $^1$H-MRS technique will not be able to answer the question of whether extramyocellular fat is utilized during exercise. Intriguing are the findings by Szczepaniak et al. (291) infusing nor-adrenaline into dogs and thereby accelerating adipocyte lipolysis. They observed a trend toward a decreased EMCL signal in the gastrocnemius and tibialis anterior muscle in dogs. In contrast, the IMCL increased significantly in both muscles. The interpretation of these findings could give support to the notion that triacylglycerol located in adipocytes between the muscle fibers may be recruited for utilization in the muscle cells.

To summarize the available studies, it does not seem entirely possible to adequately explain the differing results regarding utilization of IMTG during exercise. TG is very energy dense, and only small amounts contribute with substantial energy provision, possibly explaining the difficulties detecting these changes with biochemical determination in muscle biopsies when exercise is not very prolonged. Of note, however, are the findings that females break down more IMTG during exercise than males matched for training status. Recent investigations using $^1$H-MRS and oil red O staining of muscle biopsies, however, indicate the use of IMTG during submaximal exercise, and the oil red O technique has also shown that IMTG breakdown apparently only occurs in type 1 fibers. This can influence the biochemical IMTG measurements, especially when a mixed muscle is studied. Furthermore, it cannot be excluded that some of the LCFA taken up in the exercising muscle is esterified to IMTG in nonactive motor units, especially at lower exercise intensities, and this could influence the data obtained. Nevertheless, the contribution to energy provision in males is rather low, in the range of ~10% of total energy expenditure in the fasting state (and likely lower in the fed state), whereas the IMTG store in females seems to be more dynamic, and breakdown of IMTGs in females may cover up to 10–25% of energy provision during exercise.

E. Is Intramyocellular Triacylglycerol Breakdown During Exercise Influenced by Training?

It is controversial whether IMTG hydrolysis and utilization during exercise is increased by training. Applying the muscle biopsy technique, only a few studies have demonstrated a higher IMTG utilization during exercise in the trained compared with the untrained situation when exercise was performed by males at the same absolute intensity (133, 214), whereas other studies were unable to obtain a training-induced increase in IMTG utilization in males (14, 147, 235) or in females (281) at the same
six studies in HSL knock-out mice that exhibited normal
limiting in lipolysis (76). This view has been challenged by
somewhat difficult to compare.

(94, 141, 267) as substrates, which make the findings
168, 169, 236, 267, 323, 326, 329, 330) or cholesteryl esters
or triacylglycerol (60, 85, 161,
muscle, HSL activity has been measured using diacylglyc-
strates have been used. Thus, in adipose tissue or skeletal
for diacylglycerol and cholesteryl esters compared with
enzyme exhibits an
340). From in vitro assay systems it is evident that the
specificity that efficiently catalyzes the hydrolysis of tri-
erol esters, and retinyl esters in adipose tissue (13, 331,
HSL is a multifunctional lipase with a broad substrate
specificity that efficiently catalyzes the hydrolysis of tri-
acylglycerol, diacylglycerol, monoacylglycerol, choles-
terol esters, and retinyl esters in adipose tissue (13, 331, 340). From in vitro assay systems it is evident that the
enzyme exhibits an ~10- and 5-fold higher specific activity
for diacylglycerol and cholesterol esters compared with
triacylglycerol or monoacylglycerol, respectively (76, 77).
In assays to measure HSL activity, several different sub-
strates have been used. Thus, in adipose tissue or skeletal
muscle, HSL activity has been measured using diacylglyc-
erol as substrate (9, 267) or triacylglycerol (60, 85, 161, 168, 169, 236, 267, 323, 326, 329, 330) or cholesterol esters
(94, 141, 267) as substrates, which make the findings somewhat difficult to compare.

Studies on adipocytes have indicated that HSL is rate
limiting in lipolysis (76). This view has been challenged by
studies in HSL knock-out mice that exhibited normal
body weight and had retained 40% of triacylglycerol lipase
activity in white adipose tissue (207). Furthermore, it was
recently shown (99) that HSL-deficient mice accumulated
diacylglycerol in adipose tissue and skeletal muscle, indi-
cating that when HSL is missing this leads to an incom-
plete hydrolysis of triacylglycerol with an interruption of
the lipolytic cascade at the stage of diacylglycerol hydro-
lisis. This observation was supported by the findings of a
marked reduction in the formation of fatty acids in skel-
etal muscle as in several other tissues (99). Furthermore,
in vitro studies with HSL-deficient fat pads demonstrated
an accumulation of diacylglycerol and a drastic reduction
in fatty acid production after β-adrenergic stimulation compared with the wild type (99). Interestingly, when the
specific triacylglycerol hydrolase activity (the enzymatic
conversion of TG to DAG) was calculated, the specific TG
hydrolase activity was reduced 50% in adipose tissue,
whereas no reduction was found in skeletal muscle when
comparing HSL-deficient mice with wild type (99). These
data suggest that HSL is rate-limiting in the catabolism of
DAG but not of TG hydrolysis in skeletal muscle and
furthermore points to the existence of one or more lipases
with considerable activity, specifically to TG hydrolysis in
skeletal muscle. Further support for the existence of lipases other than HSL involved in basal triacylglycerol
hydrolysis in skeletal muscle appears from recent studies,
where neutral lipase activity in human skeletal muscle
only decreased by ~25% when antisera against HSL was
added to the assay medium (236, 329). When a similar
approach was taken in basal, resting rat soleus muscle
(169, 170), HSL activity was decreased by ~60%, indicat-
ing that HSL may be less dominating in human skeletal
muscle than in rat skeletal muscle and that more lipases
than HSL are involved in TG hydrolysis in the resting state.

A. Hormone-Sensitive Lipase Activity
During Exercise

It has been shown that electrically induced muscle
contractions increase the neutral lipase activity of soleus
muscle of rats (169). Studies in humans have likewise
demonstrated an exercise-induced increase in neutral
lipase activity in skeletal muscle (161, 236, 323, 326, 329).
In human muscle, the measured increase in neutral lipase
activity by exercise was completely abolished when mus-
cles were preincubated with anti-HSL before measuring
neutral lipase activity, clearly demonstrating that the in-
crease in neutral lipase activity during exercise was due
to an increase in HSL activity, whereas the remaining
neutral lipases were not affected by exercise (236, 329).
Similar findings have been obtained previously in rat skel-
etal muscle (169). An intriguing finding in studies where
exercise-induced muscle contractions or electrical stimu-
loration have been applied is that the effect of muscle contractions was transient, as neutral lipase activity and in some studies actual HSL activity increased rapidly at initiation of exercise or electrical stimulation, but then declined towards resting, basal values during continued exercise or electrical stimulation (169, 236, 323). The reason for this transient activation is not known. It is surprising from the point of view that exercise is accompanied by increases in circulating catecholamine concentrations, and there is evidence for activation of HSL by epinephrine. Thus, in incubated rat soleus muscle, HSL was activated by epinephrine (170). Also in human skeletal muscle, evidence was provided that β-adrenergic stimulation by infusion of epinephrine increased neutral lipase activity at rest and during exercise (161, 330). Nevertheless, the role of physiological concentrations of epinephrine in exercise-induced increased HSL activity is not clear due to the only temporary increase in muscle HSL activity with exercise despite increasing epinephrine concentrations.

B. Molecular Mechanisms

1. Phosphorylation

The molecular mechanisms behind the activation of HSL in skeletal muscle are quite incompletely known. It appears from recent studies that HSL activity, both in adipose tissue and skeletal muscle, is regulated by phosphorylation, by allosteric mechanisms, and by translocation of the enzyme to the fat droplet, although translocation has yet only been demonstrated in adipocytes (13, 85, 125, 127, 267).

With regard to phosphorylation, five phosphorylation sites on HSL have so far been identified as regulatory sites. In vitro studies have demonstrated that Ser\textsuperscript{563}, Ser\textsuperscript{659}, and Ser\textsuperscript{660} are cAMP-dependent protein kinase A (PKA) targets on HSL. In adipocytes, all three sites are phosphorylated both in vivo with isoprenaline and in vitro when incubated with PKA (9, 125). Although it is well known that the PKA sites on HSL are phosphorylated by catecholamines in adipocytes, it has not until recently been investigated in skeletal muscle (236). Interestingly, despite a severalfold increase in arterial epinephrine concentrations during exercise, phosphorylation of Ser\textsuperscript{563} on HSL in human vastus lateralis muscle was not enhanced compared with rest (236). These findings suggest that Ser\textsuperscript{563} on human skeletal muscle HSL may not be a target for PKA in skeletal muscle during exercise. Alternatively, HSL in human skeletal muscle may be constitutively phosphorylated on Ser\textsuperscript{563} by resting catecholamine levels and/or very high plasma catecholamine concentrations are needed to further increase skeletal muscle Ser\textsuperscript{563} phosphorylation during exercise. These data do not support epinephrine-induced phosphorylation of HSL on Ser\textsuperscript{563} to be of importance for regulating muscle HSL activity during exercise. So far, of the sites on HSL known to be phosphorylated by epinephrine in adipocytes, only Ser\textsuperscript{563} has been investigated in muscle.

Activation of the extracellular signal-regulated kinase (ERK) pathway was shown to increase lipolysis and HSL activity in adipocytes by phosphorylating HSL on Ser\textsuperscript{600} (94). Furthermore, it was recently demonstrated that in contracting isolated rat soleus muscle, HSL activation required the involvement of ERK and/or protein kinase C (60).

An additional phosphorylation site on HSL, Ser\textsuperscript{565}, can be phosphorylated by AMP-activated protein kinase (AMPK) and Ca\textsuperscript{2+}/calmodulin-dependent kinase II (CaMKII) (85, 205, 267, 340). When adipocytes were pre-incubated with AICAR, an AMP mimicking agent, isoprenaline-induced lipolysis was inhibited (52, 289). It was also shown that phosphorylation of HSL by AMPK prevented subsequent phosphorylation by PKA, and vice versa (85). Based on this, it was proposed that phosphorylation of Ser\textsuperscript{565} exerts an antilipolytic role (85).

AMPK is activated during exercise (38, 59, 82, 236, 284, 318, 329, 334, 336, 338), and the activation is inversely related to content of glycogen in skeletal muscle (59, 236, 329, 336). The study by Roepstorff et al. (236) is the first to investigate the effect of AMPK on HSL Ser\textsuperscript{565} phosphorylation and HSL activity in human skeletal muscle. During exercise with either high or low muscle glycogen content, low and high muscle activity of α\textsubscript{2}AMPK, respectively, was achieved. An expected finding was that in the trial with the highest muscle α\textsubscript{2}AMPK activity, the highest HSL Ser\textsuperscript{565} phosphorylation in muscle was also achieved. Nevertheless, differences were not obtained in HSL activity or IMTG hydrolysis between trials. These findings are in accordance with observations in isolated contracting rat soleus muscle, where it was shown that muscle HSL activity varied in the face of constant AMPK activity (61). In contrast, in another study it was in fact reported that AMPK activation may inhibit HSL activity during exercise, although no effect on muscle triacylglycerol breakdown was reported (329). Taken together, the data suggest that AMPK can phosphorylate HSL on Ser\textsuperscript{565} in skeletal muscle, but AMPK seems to be of minor importance as a regulator of HSL activity and muscle triacylglycerol breakdown in skeletal muscle during muscle contraction/exercise.

2. Allosteric regulation

TG hydrolysis also appears to be under allosteric regulation. In in vitro incubated purified bovine adipocytes, oleoyl CoA and oleic acid inhibited HSL activity, measured against cholesterol olate. Of these, the fatty acyl CoA was found to have the greatest effect, decreasing HSL activity by 50% at ~0.1 μM, versus 50% at ~0.5
µM with the fatty acid (141). In homogenates from resting rat soleus muscle, a ~20% reduction in neutral lipase activity was found when palmitoyl CoA was added (328). During exercise, an increase in muscle LCFA CoA content has been observed (323). Also exercise at high intensities has revealed an increased intramyocellular concentration of LCFA in human skeletal muscle (154). The cytosolic concentrations of long-chain fatty acyl CoA and of fatty acids during exercise might be sufficient to inhibit in vivo HSL activity (141), whereby TG lipolysis will be reduced. Thus it is worth considering the allosteric inhibition of TG hydrolysis in muscle tissue during exercise, and it could be speculated that the allosteric inhibition in some cases might override activating phosphorylation during exercise. In addition, phosphorylation of HSL might change the sensitivity of HSL towards its allosteric regulators.

VII. RELATION BETWEEN LIPIDS AND INSULIN RESISTANCE

A. Intramyocellular Triacylglycerols and Insulin Resistance

The content of IMTG has been proposed to be related to insulin resistance. Thus Falholt et al. (69) originally showed that in type 2 diabetic insulin-resistant patients, the triacylglycerol content in the rectus abdominis muscle was significantly larger compared with healthy individuals, and in rats it was also demonstrated that IMTG correlated negatively to insulin sensitivity (286). Later, Pan et al. (208) showed in a cross-sectional study, involving among others overweight Pima Indians [body mass index (BMI) = 37 kg/m²] an inverse relationship between whole body insulin sensitivity (determined by the euglycemic hyperinsulinemic clamp technique) and the content of muscle triacylglycerol measured in biopsies from the vastus lateralis muscle. A significant inverse relationship between insulin activation of glycogen synthase and muscle IMTG has also been described in middle-aged nondiabetic women (213). When indirect methods to measure muscle content of triacylglycerol like computed tomographic imaging (CT) and 3H-MRS have been applied (91–93, 272, 291) or morphometry and oil red O staining have been used (91, 93, 112, 175, 190), an inverse relationship between muscle content of triacylglycerol and insulin sensitivity has also been demonstrated. What characterizes these latter studies is that increased muscle triacylglycerol content and decreased insulin sensitivity was observed in either overweight, insulin-resistant individuals, with and without type 2 diabetes (91–93, 112, 175), in normal weight insulin-resistant relatives to patients with type 2 diabetes (210), or in severely obese individuals (190). A negative association between IMTG content and whole body insulin-stimulated glucose uptake was, however, also found in normal-weight, healthy individuals (BMI = 24.1–25.7 kg/m²) when IMTG was measured by the 3H-MRS technique in the soleus muscle (166, 210) or in the vastus lateralis muscle (319).

However, there are instances where the correlation between insulin sensitivity and IMTG content is not present. For instance, in healthy young male volunteers who for 4 wk consumed a diet consisting of 45 E% carbohydrates of which the carbohydrate food items were either of high or low glycemic index (HGI and LGI, respectively), the biochemical measured content of IMTG in the vastus lateralis muscle was significantly higher after the HGI diet compared with after the LGI diet and so was insulin sensitivity, measured by the euglycemic, hyperinsulinemic clamp procedure (152). Furthermore, endurance-trained subjects who have an increased sensitivity to insulin are often found to have high IMTG content (58, 91, 130), although this effect is very dependent on the diet (117, 148, 281) as discussed previously. Recently, it was reported that 8 wk of moderate exercise training in type 2 diabetic patients (BMI = 32 kg/m²) decreased IMTG content to values similar to the healthy controls, yet insulin sensitivity was still markedly lower in the trained diabetics than even untrained controls (29). Also in recent studies, performed in normal-weight, healthy, insulin-sensitive individuals, using either the 3H-MRS technique (260, 272) or the muscle biopsy technique (113), no association between insulin sensitivity and IMTG content was demonstrated. Finally, in females, we have recently described that IMTG content is higher than in males irrespective of training status (281). If intramuscular triacylglycerols are indeed directly involved in insulin resistance, one would assume that females would be insulin resistant compared with males. However, this is not the case. It has been demonstrated that women are more sensitive to insulin than similarly fit men (201).

Summarizing the available data, it is apparent that IMTG content may not be directly involved in determining insulin sensitivity but rather may be a marker of decreased insulin sensitivity in populations where lipids are stored as a consequence of an imbalance between lipid supply and lipid oxidation rate in skeletal muscle. Accordingly, lipid infusion studies in healthy male volunteers as well as fat feeding for 3 days caused insulin resistance and increases in muscle IMTG, quantified by 3H-MRS, in soleus and tibialis anterior muscles after lipid infusion, but only in the tibialis anterior muscle after the high-fat diet (10, 19). Thus a likely scenario is that when the level of physical activity and hence energy utilization is low compared with the intake of lipids, skeletal muscle may partition LCFA towards re-esterification to IMTG rather than to oxidation (Fig. 15).

From several recent studies in obese subjects and type 2 diabetic patients, it is also apparent that the intramuscular deposition of lipids is a consequence of an
imbalance between rate of LCFA uptake and rate of LCFA oxidation. Thus, in a study by Kelly et al. (144), severely obese but glucose-tolerant female and male volunteers (BMI = 34 kg/m²) were compared with healthy subjects (BMI = 23 kg/m²). Despite similar arterial LCFA concentrations and LCFA uptake in both groups, measured across the leg by infusion of [U-C¹⁴]oleic acid, a lower oxidation of fatty acids was seen in the obese group. Similarly, an increase in LCFA uptake, measured in muscle strips from the rectus abdominis muscle, was demonstrated in combination with a decrease in LCFA oxidation and increase in IMTG synthesis in extremely obese women (132), and in obese, nondiabetic women (283) compared with lean subjects. Furthermore, similar findings were obtained in insulin-resistant skeletal muscle of obese Zucker rats where rates of palmitate esterification were markedly increased concomitantly with a reduced oxidation of LCFA (304). It has been reported that LCFA transport rate and FAT/CD36 content in giant sarcolemmal vesicles prepared from human rectus abdominis muscle was upregulated approximately fourfold and associated with an increased IMTG content in obese individuals and in type 2 diabetics compared with lean subjects (25), suggesting that sarcolemmal transport capacity of LCFA is increased in obesity and type 2 diabetes. Taken together, the available evidence suggests that in obesity and type 2 diabetes decreased muscle oxidation and possibly increased transmembrane transport capacity of LCFA compared with lean individuals led to increased IMTG deposition. This picture seems to be most pronounced in severe obesity and type 2 diabetic patients rather than in moderately obese subjects.

B. Lipid Intermediates and Insulin Resistance

Several factors could be involved in the direction of LCFA or actually long-chain acyl CoA (LCA-CoA) towards esterification rather than oxidation in obesity and type 2 diabetes. Localization of mitochondria more centrally in the muscle cell will increase the transport distance for LCA-CoA to the mitochondria for oxidation, increasing the likelihood for esterification rather than oxidation. In fact, it was recently described that insulin-resistant obese and type 2 diabetic subjects displayed decreased subsarcolemmal mitochondria in the vastus lateralis muscle compared with lean controls (232). It is, however, probably more important that several findings point toward decreased mitochondrial oxidative capacity as central in the reduced oxidation rate of LCFA-CoA. Thus a number of studies have shown that the activity of the key enzymes citrate synthase (CS) and β-hydroxy acyl dehydrogenase (HAD) are significantly reduced in skeletal muscle in obesity (47, 112, 132, 145, 159) and type 2 diabetes (18, 112, 145, 270). Furthermore, the muscle activity of CPT1 was also reduced in association with obesity in a number of studies (132, 145, 159, 271). Because mitochondrial oxidative capacity is low in insulin-resistant subjects (47, 91, 145, 232, 270, 271), mitochondrial dysfunction and thereby decreased lipid oxidation has been thought to be
important in development of insulin resistance. Accordingly, a connection between mitochondrial dysfunction, increased intramuscular triacylglycerol levels, and insulin resistance has recently been described in insulin-resistant offspring of patients with type 2 diabetes (211).

Interestingly, it is not only the oxidative capacity of skeletal muscle that seems to be of importance as an increased activity of marker enzymes of the glycolytic pathway: phosphofructokinase (PFK), glyceraldehyde phosphate dehydrogenase (GAPDH), and hexokinase (HK) were observed in obese, non-insulin-dependent diabetic patients (270) compared with lean subjects, and the HK/CS ratio had the strongest correlation with insulin sensitivity. The findings suggest that a dysregulation between mitochondrial oxidative capacity and capacity for glycolysis is an important component in the mechanism of insulin resistance because such a dysregulation contributes to a decreased lipid oxidation rate and a direction towards reesterification of LCFA by providing glycerol-3-phosphate (Fig. 15).

However, the rate of reesterification of LCFA-CoA towards triacylglycerol and phospholipids is apparently not adequate to handle prolonged lipid oversupply, since increased muscle concentrations of LCA-CoA and diacylglycerol (DAG) are associated with lipid oversupply (50, 189). Interestingly, an inverse relationship between total content of LCFA-CoA in the vastus lateralis muscle and whole body insulin action was demonstrated in male individuals with varied degrees of BMI (24–38 kg/m²) and glucose tolerance (63), and in obese insulin-resistant Zucker rats, LCFA-CoA content was increased by 90% compared with lean controls (75). Likewise, in mice, overexpression of LPL led to an increase in intramuscular LCFA-CoA, DAG, and ceramide concentration in skeletal muscle in combination with muscle-specific insulin resistance (156). Nevertheless, a recent training study of type 2 diabetics showed improved insulin sensitivity after training but no change in LCFA-CoA content (29). Thus the role of LCFA-CoA in insulin resistance is not totally clear, although there are a number of identified mechanisms by which LCFA-CoA may influence glucose metabolism. For instance, studies in homogenates from human and rat soleus muscle in vitro have shown that LCFA-CoA can inhibit hexokinase activity, which may contribute to a reduction in glucose metabolism (294) (Fig. 16).

LCFA is also a substrate for the synthesis of ceramide, and increased availability of palmitate has been found to elevate intracellular ceramide levels. Thus, when mouse skeletal muscle C₆C₁₂ myotubes were incubated with saturated fatty acids, the content of cellular ceramide was elevated (257). A recent study demonstrated that LCFA fatty acid was present in human vastus lateralis muscle (114). Ceramide has been linked to insulin resistance. Hence, increased levels of ceramide have been found in insulin-resistant muscle of the Zucker rat (305). Furthermore, when C₆C₁₂ myoblasts were pretreated with palmitate or a cell-permeable ceramide analog, insulin-stimulated glycogen synthesis and Akt/PKB activation were inhibited, without affecting IRS-1 tyrosine phosphorylation (258). In addition, incubation of L6 myo-

**FIG. 16.** Mechanisms whereby intramuscular lipids may interfere with the insulin signaling pathway and glucose metabolism in human skeletal muscle. When protein kinase C (PKC) is activated by lipid intermediates, the insulin receptor is phosphorylated (p) on a serine (S) residue, whereby initiation of the insulin signaling cascade is inhibited. Besides, lipid intermediates may inhibit the insulin signaling cascade further downstream as well as early in the glucose uptake pathway. IMTG, intramuscular triacylglycerol; DAG, diacylglycerol; LCFA CoA, long-chain fatty acyl CoA; PKC, protein kinase C; IR, insulin receptor; IRS, insulin receptor substrate; (p), phosphorylation; S, serine residue; T, tyrosine residue; P3K, phosphoinositide 3-kinase; PDK, 3-phosphoinositide-dependent kinase; GSK-3, glycogen synthase kinase 3; GS, glycogen synthase; HK, hexokinase; G-6-P, glucose-6-phosphate.
cytes with palmitate caused marked ceramide synthesis and decreased insulin sensitivity involving impaired activation of Akt/PKB (104, 218). Nevertheless, despite the evidence for effects of ceramide on insulin action, infusion of intralipid caused insulin resistance in humans but no increased muscle ceramide concentrations (137). Furthermore, a single exercise bout, which is known to increase insulin sensitivity (229), apparently increased rather than decreased muscle ceramide concentrations (114). Finally, treatment of obese Zucker rats with rosiglitazone increased glucose tolerance but also increased muscle ceramide levels (174). Thus further studies are needed to clarify the potential role of ceramides in insulin resistance.

C. Protein Kinase C

LCFA-CoA may interfere with the insulin signaling pathway directly by activating protein kinase C (PKC) (Fig. 16). The PKC family constitutes a group of multifunctional serine/threonine protein kinases involved in metabolism, mitogenesis, and gene expression (70, 247). Depending on mode of activation, the PKC family is divided into three subgroups. Conventional isoforms (α, β1, β2, γ) are dependent on both Ca$^{2+}$ and DAG for stimulation of activity, novel isoforms (δ, ε, θ, η) are dependent on DAG, and the atypical isoforms (ζ, λ, ι, where PKC-ι is the mouse homolog of human PKC-ι) that are independent on Ca$^{2+}$ and DAG but are activated by phosphatidic acid and phosphatidylinositol 3,4,5-trisphosphate (PIP$_3$) (247). During activation, the kinases can translocate within the cell, and the extent to which a PKC isozyme is found in the membrane fractions is commonly used as a measure of its activation. It is believed that PKC inhibits insulin signaling by phosphorylation of the serine residues on the insulin receptor (138, 292) and insulin receptor substrate-1 (IRS-1) (227) in turn inhibiting the tyrosine phosphorylation of IRS-1, leading to decreased insulin-stimulated phosphatidylinositol 3-kinase activity (95, 341) (Fig. 16). In this respect, it appears that some conventional and in particular some of the novel PKC isoforms are involved in downregulating the insulin signaling cascade (95, 341). Recently, it was shown that PKC-θ can phosphorylate IRS-1 at Ser-1101 and block tyrosine phosphorylation of IRS-1 and downstream activation of the Akt pathway (176).

From studies in rats where the plasma concentration of LCFA was acutely elevated by lipid infusion for 5 h and insulin resistance was induced, an increased membrane localization of PKC-θ, and thereby presumably activation of the isozyme, was found concomitantly with a decrease in the cytosol (95, 341), whereas no changes were observed in PKC-ε (341). When insulin resistance and increased lipid levels were obtained by prolonged glucose infusion in rats, PKC-ε translocation to the membrane fraction was observed (173). In insulin-resistant obese Zucker rats, the membrane localization of both PKC-ε and -θ was increased (222), and the PKC-ε expression was increased in muscle from a diabetes prone-line sand rat (Psammomys obesus) (266). Furthermore, PKC-θ knock-out mice are protected from fat-induced insulin resistance (157), further supporting an important role of novel PKCs in development of lipid-induced insulin resistance. In addition to the acute effect of elevated plasma LCFA levels, chronic effects of high fat feeding for 3 wk appear to activate both PKC-θ and -ε, which was associated with insulin resistance (256).

The mechanism by which LCFA-CoA causes activation of PKC likely involves accumulation of DAG and activation of the DAG-sensitive isoforms of PKC. DAG can be generated through esterification of LCFA-CoA towards muscle triacylglycerol, from hydrolysis of muscle triacylglycerol and by breakdown of phospholipids particularly phosphatidylinositol 4,5-bisphosphate and phosphatidylcholine by the action of phospholipases C and D, respectively (164) (Fig. 16). Cell culture studies indicate that DAGs with a saturated fatty acid profile generally are poor PKC activators, whereas DAGs with an unsaturated fatty acid profile are better activators of DAG-sensitive PKC isoforms (123, 251, 321). Studies implying a role for DAG in lipid-induced insulin resistance include the study by Yu et al. (341) in which intralipid plus heparin infusion in rats induced an increase in intracellular levels of LCFA-CoA, and a transient increase in DAG concomitantly with increased activation of the novel PKC isozymes θ and ε. An increase in DAG associated with translocation and activation of PKC-θ and -ε was also found in obese Zucker rats (51, 222). Thus DAG levels in skeletal muscle of rats have been found to be elevated in several situations of lipid-induced insulin resistance as well as in transgenic mice overexpressing LPL in muscle (156) and in several cases in association with increased activity of PKC-θ and -ε (222, 255, 341). Thus studies in rats, using different models, strongly support a link between activation of especially PKC-ε and -θ, serine phosphorylation of IRS-1, and in turn decreased phosphatidylinositol 3-kinase activity and decreased insulin sensitivity by increased lipid availability to skeletal muscle. However, one study does not fit in this picture: when obese Zucker rats were treated with rosiglitazone, which improved glucose tolerance, skeletal muscle IMTG, DAG, and ceramide content were increased rather than decreased, and translocation of novel PKC isoforms to the membrane fraction of muscle was not observed (174). It is likely that roziglitazone increases glucose tolerance by other mechanisms that can override the detrimental effects of lipid accumulation in muscle.

Patients with type 2 diabetes have increased PKC-θ protein levels and isozyme activity in the rectus abdomi-
nus muscle (136) and decreased muscle insulin receptor tyrosine kinase activity, which could be restored by phosphatase treatment in vitro, possibly suggesting increased serine phosphorylation of the insulin receptor due to increased PKC activity (138). In only one human study in healthy, lean male subjects the association between insulin resistance, caused by acute intralipid infusion for 5 h, and muscle PKC isozyme content has been examined (137). In this study, elevated plasma LCFA was associated with increased DAG mass and an increase in membrane PKC-δ protein and membrane PKC-βII, whereas no significant changes were observed in PKC-ε, -θ, or -ζ measured in the vastus lateralis (137). These findings in humans are in contrast to findings in rats using the same model for generating insulin resistance, where an activation of PKC-θ has been found. However, in the human study by Itani et al. (137), muscle samples were not obtained until after 6 h of intralipid infusion, and it cannot be excluded that PKC-θ was activated at an earlier or perhaps later stage. Supporting the latter assumption, obese diabetic patients showed a significantly higher PKC-θ content and activity in the rectus abdominus muscle compared with muscles from obese, normoglycemic controls (138).

In summary, lipid oversupply in relation to lipid expenditure seems to result in decreased insulin action in muscle by a variety of mechanisms involving accumulation of IMTG, LCA-CoA, and DAG and activation of PKC and possibly synthesis of ceramide. The net result is a decrease in insulin signaling and decreased glucose uptake (Fig. 16). Exercise would be expected to be particularly beneficial in preventing these events by increasing the oxidation of lipids and thereby partitioning lipids towards oxidation rather than reesterification and accumulation. Whereas studies have shown increased insulin sensitivity after training in obese or type 2 diabetic subjects improvements in IMTG and LCFA-CoA content in muscle were not consistently seen (29, 83, 111). Thus the relation between IMTG and LCFA-CoA and insulin resistance is not clearly apparent when exercise is used as an intervention to increase insulin sensitivity.

VIII. CONCLUSION AND FUTURE DIRECTIONS

It is evident that the control of lipid utilization in skeletal muscle is exquisitely intricate and is subject to a plethora of regulatory mechanisms that have different degrees of importance at rest and during or after exercise. Recent evidence has implicated the lipid binding proteins as regulators of transmembrane transport of LCFA, and intriguingly, caveolin-3 has been found to colocalize with FAT/CD36 in muscle. Unraveling the possible role of caveolae and caveolins in lipid transport will be an important further step in our knowledge of lipid metabolism also in exercise. Still, the importance of fatty acid transporters in limiting utilization of LCFA during exercise remains unsettled, and regulatory steps in mitochondrial access of the lipid may seem to be more important. In this respect, a major regulatory role for carnitine in controlling lipid oxidation during exercise is proposed. Clearly, further research is needed to clarify the relative importance of the various regulatory steps in lipid oxidation during exercise.

The metabolic role of LPL has been studied in various tissues; however, the role of LPL in muscle lipid and lipoprotein metabolism at rest, during, and after exercise is an important aspect of current and future research. Especially the mechanisms involved in posttranscriptional, translational, and posttranslational processes that regulate LPL maturation and enzyme translocation to the capillaries in skeletal muscle should be elucidated. IMTG utilization during exercise depends on preexercise diet, gender, training status and duration, intensity, and mode of exercise. The available methods for measuring IMTG all have their limitations. Because IMTG utilization is fiber type specific, the fiber type recruitment pattern during exercise is of importance when measuring IMTG utilization in muscles with mixed fiber type distribution. However, the contribution of IMTG for energy provision may not be large, covering ~10% of energy provision in fasting exercise in male subjects, whereas in females IMTG may cover a larger proportion of energy delivery. A further step is to elucidate how IMTG turnover during exercise is regulated at the molecular level and in that respect regulation of HSL activity on the molecular level during exercise is now in progress.

Finally, lipids are heavily implicated in development of insulin resistance in skeletal muscle. This seems to be linked to an imbalance between lipid supply and lipid oxidation, the latter being related to decreased mitochondrial oxidative capacity in states of insulin resistance. Because exercise has the ability to both increase lipid oxidation and increase muscle mitochondrial oxidative capacity, it will be important to further establish the molecular mechanisms behind lipid-induced insulin resistance as well as the role of exercise in preventing and treating this condition.

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