Lipase regulation of muscle triglyceride hydrolysis

LAWRENCE B. OSCAI, DAVID A. ESSIG, AND WARREN K. PALMER
Exercise Research Division, Department of Physical Education,
University of Illinois at Chicago, Chicago, Illinois 60680

OSCAI, LAWRENCE B., DAVID A. ESSIG, AND WARREN K. PALMER. Lipase regulation of muscle triglyceride hydrolysis. J. Appl. Physiol. 69(5): 1571-1577, 1990.—The cellular control of intramuscular triglyceride (TG) metabolism involves two major identified lipases: hormone sensitive lipase (HSL) and lipoprotein lipase (LPL). Recently, the presence of HSL in muscle has been unequivocally demonstrated. However, although it is thought that HSL is responsible for intramuscular TG lipolysis, direct evidence for this is lacking. There is evidence to suggest that HSL and LPL are simultaneously activated under a variety of conditions. The two muscle lipases appear to be turned on by the same signal and function as a coordinated unit in meeting the energy demands of muscle. At a time when HSL is presumably hydrolyzing endogenous TG, LPL is sent to the capillary beds in search of substrate. TG uptake from circulation is highly related to muscle LPL activity. Exercise training increases LPL activity in plasma and in parenchymal cells in muscle. These results suggest that training may increase the capacity to clear TG from circulation and that LPL might have a role in replenishing muscle TG stores that have been decreased with exercise.

hormone-sensitive lipase; lipoprotein lipase; muscle lipolysis; exercise

FATTY ACIDS are the preferred substrate for oxidative metabolism in the isolated beating heart (34, 35). Fatty acids actually inhibit glucose uptake, glycolysis, and glycogenolysis in the heart (34). Randle et al. (46) have proposed that the amount of fatty acids available to the cell determines the level of suppression of carbohydrate utilization. In this context, an increased availability of fatty acids has been shown to inhibit carbohydrate utilization in skeletal muscle of exercising rats (48). Utilization of fat and carbohydrate is related to the intensity of the work (23). When heavy aerobic exercise [i.e., ≥75% maximal O2 consumption (VO2 max)] is performed, glycogen appears to be an indispensable energy source. However, when glycogen stores are reduced, prolonged exercise can still be performed at submaximal levels (i.e., ≤60% VO2 max) if the supply of fatty acids is adequate (45). These results provide evidence that the oxidation of fat can supply almost all the energy required by working muscle during light-to-moderate exercise.

Triglycerides (TG), the storage form of fatty acids in muscle, are located in two distinct pools: a pool found in circulation and an intracellular pool found within the muscle cell. This review focuses on the enzymatic mechanisms involved in the regulation of muscle TG hydrolysis in these two pools. Evidence is presented to show that hormone-sensitive lipase (HSL) is present in muscle and that this lipase may be responsible for intracellular lipolysis. Furthermore, correlations between intracellular lipolysis and lipoprotein lipase (LPL) activity suggest a coordinate regulation of HSL and LPL in muscle. Evidence is also presented to show that LPL may have an important role in replenishing tissue lipid stores. Because our interest revolves around fuel provision to muscle during exercise, the interaction of exercise and fat metabolism is emphasized when appropriate.

Sources of Fat

The heart contains a pool of endogenous TG present in interstitial fat cells (cardioadipocytes) or as free-floating cytosolic droplets, membrane-bound particles, or lipid-filled vacuoles. Figure 1, an electron micrograph of a longitudinal section of rabbit left ventricle, shows intracellular lipid droplets. Skeletal muscle also contains lipid droplets of the type shown in Fig. 1. It has been estimated that ~50% of the fat oxidized during exercise comes from intramuscular TG stores (4, 19, 47). Endurance-trained individuals oxidize proportionately more fat and less carbohydrate at any given relative work rate.
FIG. 2. Model for muscle free fatty acid (FFA) metabolism and a proposed mechanism for regulation of hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL) mediated triglyceride (TG) hydrolysis in muscle.

FIG. 1. Left ventricular muscle (rabbit) showing existence of numerous lipid droplets (L). Note proximity of lipid droplets to mitochondria. Bar, 1 μm. ×10,000. (Courtesy of B. R. Eisenberg, Dept. of Physiol. and Biophys., Univ. of Illinois at Chicago.)

Metabolism of Fatty Acids in Muscle

An overall model for the transport and metabolism of fatty acids in muscle has been proposed (51, 63). Fatty acids originating from chylomicrons and very-low-density lipoproteins are hydrolyzed by LPL (Fig. 2). These fatty acids are taken up by muscle, esterified in the sacroplasmic reticulum to TG, and incorporated into lipid droplets in muscle. The total amount of fatty acids in muscle available for oxidation represents the sum of those in the free form plus those in the esterified form. The available data indicate that 70-90% of the fatty acids entering muscle cells from circulation are rapidly esterified to TG (30, 57). The remaining fatty acids entering the cell form a pool of nonesterified fatty acids (30, 58, 59). The fatty acids released through intracellular lipolysis are transported to the mitochondria for β-oxidation. Terjung et al. (58, 59) have shown that exercise increases the muscle’s free fatty acid pool about threefold and decreases the TG pool 34%. Thus, exercise increases the availability of intracellular nonesterified fatty acids to working muscle.

Regulation of Intramuscular Lipolysis

In 1964, Williamson (60) demonstrated that epinephrine caused increased glycerol release in the isolated perfused heart. Several years later, Gartner and Vahouny (14) showed that TG were decreased ~50% in rat heart perfused with dibutyryl adenosine 3′,5′-cyclic monophosphate (cAMP). These results indicated that cardiac lipolysis was mediated through the classical cAMP cascade similar to that in adipose tissue. These early observations set the stage for numerous studies attempting to identify a hormone-sensitive cardiac TG lipase (for review, see Refs. 29 and 55).

LPL and HSL

Our knowledge about the regulation of LPL activity has increased greatly in recent years. According to Semb and Olivecrona (53), the LPL monomer is synthesized on membrane-bound ribosomes and is translocated into the lumen of the sarcoplasmic reticulum. Core glycosylation precedes final folding of the peptide with formation of an apparent S-S bridge between cysteines 283 and 278. At this stage the lipase subunits form dimers that are catalytically active. Some of the lipase molecules move rapidly to the cell surface where they can then be released into the extracellular space. When they pass the Golgi, two of their three oligosaccharides are processed into complex chains. Other lipase molecules are retained much longer in the sarcoplasmic reticulum. Whether they ever reach the processing apparatus in the Golgi or are degraded is not clear. It has been demonstrated that more than half of newly synthesized lipase molecules are rapidly degraded (52).

LPL is found in at least two fractions in muscle (3, 49). One fraction, which is readily released from heart
and skeletal muscle by perfusion with heparin, is located on the luminal surface of endothelial cells of capillaries, where it is directly involved in the hydrolysis of plasma TG. The second fraction remains in tissue after perfusion with heparin. In preliminary experiments with exercise, it was noted that free fatty acid levels were elevated in the intracellular fraction of muscle at a time when LPL activity was elevated. This suggested to us that regulation of intramuscular lipolysis may be mediated by intracellular LPL. At the time, there was no reason to believe that HSL even existed in muscle. A lipolytic role for LPL in muscle cells was strengthened by two findings. First, hormone sensitivity of the heparin-nonreleasable LPL (intracellular fraction) was demonstrated in homogenates of hearts previously treated with glucagon (38) and epinephrine (42, 43). The actions of these hormones on intracellular LPL were shown to be mediated by cAMP through protein kinase. Second, a tight inverse relationship was shown to exist between endogenous LPL activity and muscle TG concentration under a variety of biochemical (42), pharmacological (32), and physiological conditions (31). While experimentation on LPL continued in our laboratory, other investigators focused their attention on HSL in cardiac muscle (29, 55). Because epinephrine promoted lipolysis in both muscle and adipose tissue, they assumed that the muscle TG lipase must have a neutral pH optimum similar to the HSL known to reside in fat cells. This hypothesis was strengthened by the many parallels that had been shown to exist between the actions of lipolytic agents and inhibitors in adipose tissue and muscle (29). Recently, Holm et al. (24) raised an antibody to purified rat adipose tissue HSL. Using Western blotting, these investigators were able to show quantitatively the existence of an antigenic protein in muscle extract that corresponded in size (84 kDa) to the HSL in adipose tissue. The development of an anti-HSL antibody permitted quantitative enzyme measurement for the first time. In a separate set of experiments, Holm et al. (25) used the antibody to IISL to screen a cDNA expression library and to obtain a specific cDNA clone to rat adipose tissue HSL. Using the cDNA clone to perform Northern blotting experiments, they were able to provide evidence that IISL mRNA in heart and skeletal muscle was similar in size to that found in adipose tissue. Small et al. (56) took the next important step in the unfolding story of muscle HSL by simultaneously activating and phosphorylating purified HSL from bovine heart with cAMP-dependent protein kinase. However, the possibility existed that HSL purified from bovine heart could have originated from adipocytes in the interstitial spaces between cardiac cells. Therefore, in additional experiments, Small et al. (56), using HSL antibody, identified and phosphorylated IISL in rat cardiac myocyte preparations. The above results support earlier claims by Goldberg and Khoo (15) and Heathers et al. (20) that HSL can be activated in muscle with cAMP or cAMP-dependent protein kinase.

**Localization of Lipase Enzymes**

A NH4-terminal sequence of 16–30 amino acids (signal peptide) initiates transport of secretory proteins across the endoplasmic reticulum (11). Once inside the lumen of the endoplasmic reticulum, the signal peptide is cleaved from the protein. The process of secretion proceeds with further protein modification such as glycosylation, and the mature protein is then exported to the Golgi and carried forward in the transport vesicles to exit the cell. In the case of lipase enzymes, LPL mRNA encodes a NH4-terminal signal peptide of 27 amino acids (61), whereas the HSL mRNA does not appear to code for a signal peptide (25). Newly synthesized proteins lacking a signal peptide are left behind, permanent residents of the cytosolic compartment. In order for LPL to serve two functions, one inside the cell and one in circulation, two isoforms of LPL must exist. A large number of LPL cDNA clones have been isolated from a variety of different species including human, bovine, guinea pig, chicken, and mouse (8, 13, 28, 54, 61). To date, there is no evidence for two isoforms of LPL. Although different sizes of mRNA have been detected, these differences can be explained by different placement of the poly A tail on mRNA. Therefore the observation that only one isoform of LPL mRNA has been found provides strong evidence for only one LPL protein. The finding that LPL has a signal peptide, coupled with the observation that muscle contains HSL, which lacks a signal peptide, provides strong suggestive evidence that HSL and not LPL hydrolyzes intramuscular TG.

**Simultaneous Activation of LPL and HSL in Muscle**

There is evidence to suggest that LPL and HSL are activated simultaneously to provide fatty acids as muscle fuel. Figure 3 shows the pattern of reduction of endogenous TG in response to perfusion of the isolated heart for 30 min with 1 μM epinephrine. The assumption is made that HSL was responsible for muscle TG hydrolysis in this experiment because direct measurement of this enzyme's activity was not made. Figure 3 also shows that while TG were decreasing, heparin-nonreleasable LPL activity was increasing. These results suggest that a simultaneous activation of HSL and LPL occurs in muscle in response to epinephrine treatment.
A simultaneous activation of the two lipases appears also to take place under normal physiological conditions. Figure 4 shows a decrease in TG content in rat heart under conditions of fasting, fat feeding, and cold exposure. Again, we presume that HSL is responsible for muscle lipolysis. When TG were decreased, LPL was elevated. The same relationship between the two muscle lipases and the concentration of TG can be seen under conditions of exercise (39). Thus it appears that the two lipases may function as a coordinated unit in meeting the energy demands of muscle. A possible scheme for the coregulation of HSL and LPL activities is presented in Fig. 2. There is evidence that both lipases are regulated posttranslationally through the cAMP cascade (42, 56). The activation of HSL results in the hydrolysis of intracellular TG, providing fatty acids for β-oxidation. Presumably in response to the same signal (i.e., cAMP), LPL activity is also increased. The mechanism of LPL activation is both transcriptional (6, 50) and posttranscriptional (12, 50) through modification of one or more of the steps involved in synthesis, transport, and secretion of the enzyme. The exported LPL found in the capillary beds hydrolyzes circulating TG, providing fatty acids for the replenishment of lipid stores and, to a lesser extent, immediate substrate for muscle energy needs.

**Plasma TG as an Energy Source**

Prolonged exercise can result in a lowering of fasting plasma TG concentration (1, 16, 22, 41). Figure 5 shows that repeated exercise on successive days brings about a progressive decline in elevated TG levels (16, 41). The lowering of TG is seen immediately after work if the exercise is vigorous over an extended period of time, such as rats swimming to exhaustion (47), cross-country skiing for 9 h (5), or army recruits marching for 2 h (37). Even though plasma TG stores are lowered immediately after exercise, the actual energy contribution to the work performed appears to be relatively small. For example, Nikkiila and Konttinen (37) studied 40 army recruits who were fed 55 g of fat after an 11-h fast. Two hours later, 20 men marched 16 km carrying a weight of 12.6 kg over a period of 2 h and the 20 remaining men rested in bed. At the end of the experimental period the serum TG concentration in the exercise group was 126 mg/100 ml compared with 175 mg/100 ml for the control group. This difference reflects a TG utilization of ~2.45 g, corresponding to 22 kcal/2 h. With the assumption of an O₂ consumption value of 1 l/min during the march, the total caloric expenditure was ~600 kcal. Therefore, circulating TG provided ~4% of the energy required for the work. These estimations suggest that plasma TG provide a small amount of energy to muscle during vigorous work. However, it should be pointed out that plasma free fatty acid levels were nearly twice as high in the exercisers as in the controls at the end of the 2-h march. Therefore, TG synthesis could have taken place in the livers of the exercisers. Hepatic TG synthesis would serve to underestimate the contribution of plasma TG to the total amount of energy expended during the march. With milder forms of exercise, the decrease in plasma TG is not seen immediately after exercise. Instead the decrease appears 30 min to a few hours after exercise has stopped (16, 62). The exercise-induced lowering of TG has been shown to persist for 1–5 days (Fig. 5) (16, 22, 41). The lowering of TG could be attributable to a specific effect of exercise on lipid metabolism or to a decreased availability of substrate for TG synthesis secondary to the increased energy expenditure. Gyntelberg et al. (16) provide evidence that plasma TG are reduced by exercise despite an increased food intake, indicating that the TG-lowering effect of exercise is not mediated by a negative caloric intake.
Replenishment of Tissue TG Stores

There is evidence that LPL has an important role in the replenishment of TG stores in muscle. LPL activity is very low in tissues of mice born with genetic combined lipase deficiency (cld/cld) (2). Mice with this defect develop lethal hyperchylomicronemia within 2 days postpartum as a consequence of nursing (2, 44). Plasma TG values in affected mice often reach 20,000 mg/dl (100 times higher than in normal littermates) (44). Of interest is the finding of Blanchette-Mackie et al. (2) that myocytes of heart and diaphragm from suckled cld/cld mice contained no lipid droplets, whereas those from suckled unaffected mice contained numerous lipid droplets. Thus the large amount of chylomicrons in capillaries and the small amount of lipid droplets in cells of suckled cld/cld mice reflect the very low level of LPL activity in these animals. LPL appears to have an important role in providing fatty acids for intracellular TG synthesis in muscle.

As mentioned earlier, exercise decreases TG stores in muscle (4, 19, 47). The question is whether plasma LPL has a role in replenishing the decreased lipid stores in muscle. It is known that the uptake of TG from circulation is highly related to muscle LPL activity (30, 57, 59). In this context, muscle LPL activity is markedly increased with exercise training (36, 39). This exercise-induced increase in muscle LPL activity could provide an explanation for the prolonged reduction in plasma TG seen with exercise in Fig. 5. It follows then that the increased clearance of TG from circulation may provide fatty acids for the restoration of muscle TG stores reduced by exercise.

In rats not subjected to exercise, plasma LPL amounts to ~1% of that which can be released into circulation by injection with heparin (7). In other words, most of the LPL that has direct access to plasma TG is attached to the capillary walls. In contrast, skeletal muscle of exercise-trained rats killed immediately after work has very low levels of LPL attached to the capillary walls compared with that of controls (L. Oscai, unpublished observations). These results indicate that exercise has a heparin-like effect on capillary bound LPL. Because of this heparin-like effect, plasma LPL was ninefold greater in the trained than in the control animals. It seems likely that the increase in enzyme activity found in plasma is the result of the exercise-induced increase in LPL activity found inside the muscle cell (39). There is evidence that plasma LPL can hydrolyze plasma TG. For example, Hahn (17) found that when dogs with marked lipemia were injected with heparin, the lipemia disappeared completely in the blood sample taken 3–5 min later. This early finding led to the discovery of LPL. More recently, Rennie et al. (48) fed rats 5 ml of corn oil by stomach tube. Three hours later, when their plasma was visibly lipemic, the animals were given heparin sodium (200 U sc). Administration of heparin to the corn-oil-fed animals resulted in an increase in plasma fatty acid concentration to more than twice the control value after 10 min and to more than four times the control value after 40 min. These results provide evidence that circulation can become an active site for TG hydrolysis when LPL is released into the bloodstream by heparin administration. Another example of tissue TG restoration has been reported by Craig et al. (9). They provide evidence that fat cells of exercise-trained animals adapt for rapid replenishment of energy stores. For example, Craig et al. (10) show that fat cells of sedentary animals had a volume 180% greater than those of the trained animals. After cessation of training, a very rapid increase in fat cell size occurred; this increase was statistically significant after only 4 days. After 9 days without exercise, approximately two-thirds of the initial difference in fat cell volume between the trained and the sedentary rats had disappeared. A possible mechanism mediating this enhanced restoration of fat cell size could be an elevation in plasma LPL resulting from the heparin-like effect of exercise on capillary bound enzyme.

Summary

Over the past 10 years, a number of papers have been published from our laboratory suggesting that LPL might be responsible for intracellular TG hydrolysis in heart and skeletal muscle (31, 32, 38, 39, 42, 43). This stand is no longer tenable in view of recent molecular studies on LPL an the clear demonstration of a HSL in muscle identical to that in adipose tissue. We have reinterpreted our previously found correlations between intracellular lipolysis and LPL activity in terms of a coordinate regulation of HSL and LPL.

These two lipases appear to be activated simultaneously under a variety of biochemical (Fig. 3) (38, 42), pharmacological (32), and physiological (31) conditions in muscle. These results raise the possibility that both lipases are turned on by the same signal. This idea fits with the concept that HSL and LPL function as a coordinated unit that is responsible for meeting the energy demands of muscle. Both LPL (38, 42, 43) and HSL (56) appear to be regulated, in part, through the classic cAMP cascade in muscle. However, the functions of these lipases are different. At a time when HSL is apparently hydrolyzing intracellular TG, LPL is sent to the capillary beds in search of substrate.

TG stores in muscle are decreased with exercise (4, 19, 47). There is suggestive evidence that plasma TG are used to replenish the decreased fat stores in muscle. First, LPL-deficient mice (cld) develop severe hyperchylomicronemia because of an inability to clear TG from circulation (2, 44). These affected mice have no intracellular lipid droplets in the heart and diaphragm, whereas unaffected mice have numerous lipid droplets in their muscle cells. These results suggest a role for LPL in the replenishment of muscle lipid stores. Second, TG uptake from circulation is highly related to muscle LPL activity (30, 57, 59). Exercise training increases LPL activity inside muscle cells and in plasma. The finding that exercise increases the capacity to clear TG from circulation provides suggestive evidence that LPL is involved in the restoration of muscle TG stores reduced by exercise.

The assistance of Vinod Singh is gratefully acknowledged.

This research was supported by National Institutes of Health Re-
search Grants HL-38037 and AR-39872 and by the American Heart Association of Metropolitan Chicago.

Address for reprint requests: L. Oscai, Dept. of Physical Education (m/c 194), University of Illinois at Chicago, Box 4348, Chicago, IL 60680.

REFERENCES


36. Oscai, L. B., J. A. Patterson, D. L. Bogard, R. J. Beck, and...
60. WILLIAMSON, J. R. Metabolic effects of epinephrine in the isolated, perfused rat heart. I. Dissociation of the glycogenolytic from the metabolic stimulatory effect. J. Biol. Chem. 239: 2721–2729, 1964.