[Frontiers in Bioscience 3, d1011-1027, September 15, 1998]

THE REGULATION OF CARBOHYDRATE AND FAT METABOLISM DURING AND AFTER EXERCISE

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Received 9/2/98 Accepted 9/8/98

TABLE OF CONTENTS

1. ABSTRACT

The rate of carbohydrate utilization during prolonged, strenuous exercise is closely geared to the energy needs of the working muscles. In contrast, fat utilization during exercise is not tightly regulated, as there are no mechanisms for closely matching availability and metabolism of fatty acids to the rate of energy expenditure. As a result, the rate of fat oxidation during exercise is determined by the availability of fatty acids and the rate of carbohydrate utilization. Blood glucose and muscle glycogen are essential for prolonged strenuous exercise, and exhaustion can result either from development of hypoglycemia or depletion of muscle glycogen.

Both absolute and relative (i.e. % of maximal O_2 uptake) exercise intensities play important roles in the regulation of substrate metabolism. The absolute work rate determines the total quantity of fuel required, while relative exercise intensity plays a major role in determining the proportions of carbohydrate and fat oxidized by the working muscles. As relative exercise intensity is increased, there is a decrease in the proportion of the energy requirement derived from fat oxidation and an increase in that provided by carbohydrate oxidation. During moderately strenuous exercise of an intensity that can be maintained for 90 minutes or longer (~55-75% of VO_2max), there is a progressive decline in the proportion of energy derived from muscle glycogen and a progressive increase in plasma fatty acid oxidation.

The adaptations induced by endurance exercise training result in a marked sparing of carbohydrate during exercise, with an increased proportion of the energy being provided by fat oxidation. The mechanisms by which training decreases utilization of blood glucose are not well understood. However, the slower rate of glycogenolysis can be explained on the basis of lower concentrations of inorganic phosphate (Pi) in trained, as compared to untrained, muscles during exercise of the same intensity. The lower Pi level is a consequence of the increase in muscle mitochondria induced by endurance exercise training.

A large increase in muscle glycogen concentration, far above the level found in the well-fed sedentary state, occurs in response to carbohydrate feeding following glycogen depleting exercise. It was recently found that this muscle "glycogen supercompensation" is markedly enhanced by endurance exercise training that induces an increase in the GLUT4 isoform of the glucose transporter in skeletal muscle.

2. INTRODUCTION

Blood glucose and muscle glycogen are essential for prolonged, strenuous exercise. The rate of carbohydrate utilization during prolonged exercise is closely geared to the energy needs of the working muscles. In contrast, fat utilization during exercise is not tightly regulated, as there are no mechanisms for closely matching the availability and metabolism of fatty acids to the rate of energy expenditure. As a consequence, the rate of fat oxidation during exercise is determined by the rate of carbohydrate utilization and by the availability of fatty acids. The primary role of fat during vigorous exercise in humans and rodents is to spare blood glucose (i.e. liver glycogen) and muscle glycogen.

This review focuses on the regulation of carbohydrate metabolism during and after exercise and its interaction with fat metabolism. It deals primarily with studies on humans and rats. Many elegant studies have been performed on the regulation of substrate metabolism

during exercise in dogs, and the large literature on this subject has been reviewed by others (1,2).

3. REGULATION OF PLASMA SUBSTRATE UTILIZATION DURING EXERCISE

Exercise rapidly stimulates glucose transport activity in the plasma membrane (sarcolemma) of the working muscles, resulting in an increase in the influx of glucose into the cytosol (3-5). This phenomenon, which is normally the rate-limiting step in muscle glucose metabolism during exercise, is discussed in detail later in this review. Blood flow, and thus glucose delivery to the working muscles, also increases at the onset of exercise. Because glucose transport across the sarcolemma follows Michaelis-Menten, i.e. saturation, kinetics, the rate of glucose uptake at any level of sarcolemmal permeability is a function of the glucose concentration in the interstitial space, adjacent to the sarcolemma, when glucose levels are in the physiological range. If blood flow and glucose delivery did not rise to match the increase in glucose uptake by the working muscles, the glucose concentration in the interstitial space would fall markedly, preventing glucose uptake by the muscles from increasing in proportion to the rise in sarcolemmal permeability to glucose. We know of no good evidence that this occurs under normal physiological conditions, although the possibility that blood flow might become limiting under some exercise conditions can not be ruled out at our present state of knowledge. Plasma insulin concentration normally falls to a low level during exercise (6). However, under artificial, experimental conditions, when insulin is infused to raise plasma insulin, as for example in the euglycemic, hyperinsulinemic clamp procedure, or in the perfused rat hindquarter preparation, glucose delivery can become limiting (7,8)

Despite the large increase in glucose utilization during prolonged, moderate intensity exercise in the post-absorptive state, the concentration of glucose in the blood stays remarkably constant, until liver glycogen stores become depleted (9-12). How long an individual can exercise at a given exercise intensity before becoming hypoglycemic in the fasting state depends in large part on the initial liver glycogen store which, in turn, is determined by the duration of the fast and by diet composition prior to the fast (13-15). Hypoglycemia is, of course, prevented if sufficient carbohydrate is taken in during the exercise (16), and very prolonged, vigorous exercise, such as long-distance bicycle racing is possible only in the (continuously) fed state.

In the fasting state, hepatic glycogenolysis and gluconeogenesis are responsible for maintaining blood glucose levels. Gluconeogenesis, primarily from lactate and alanine produced by the muscles and glycerol produced from lipolysis, helps to spare liver glycogen and delay development of hypoglycemia (17,18). The increase in hepatic glycogenolysis that prevents development of hypoglycemia during prolonged exercise is mediated by increases in glucagon and catecholamine production and a decrease in insulin secretion (12,19-21). The increase in glucagon and the decrease in insulin appear to be the primary factors responsible for protecting against hypoglycemia, with catecholamines serving as the second line of defense (12,19-22). The evidence for this is that blood glucose is maintained despite pharmacological blockade of the adrenergic receptors (19). Catecholamines serve as a second line of defense if insulin levels are prevented from falling and glucagon levels are prevented from increasing (19). An exception is very strenuous exercise, during which it appears that afferent neural feedback from the working muscles results in the stimulation of hepatic glucose production by the sympathetic nervous system, resulting in an increase in plasma glucose (23,24). The mechanisms by which glucose production is geared so closely to glucose utilization to maintain blood glucose concentration constant during prolonged moderate intensity exercise have not yet been elucidated despite considerable research on this topic.

Oxidation of fatty acids (FFA) can provide much of the energy for prolonged moderate intensity exercise. Under normal physiological conditions the extent of fat utilization and carbohydrate sparing during exercise depends on plasma FFA concentration (25), relative exercise intensity (26-28), carbohydrate availability (29-32) and training status (33-36). Maximal rates of plasma fatty acid oxidation are attained at a relatively low exercise intensity requiring ~40% of VO_2max (27,37,38). An individual's plasma FFA concentration at a given exercise intensity is largely determined by nutritional status (17,27,29,30,33,35,39,40) and duration of exercise (41). Eating carbohydrate lowers plasma FFA both by increasing insulin levels and by providing glycerol phosphate for fatty acid resterification and storage as triglyceride (16,29,42,43). Thus, eating a diet low in carbohydrate raises plasma FFA and results in carbohydrate sparing, but is counterproductive because it reduces glycogen stores (44,45) and, therefore, endurance (39). Similarly, prolonged fasting results in a progressive rise in plasma FFA but also has the disadvantage of reducing glycogen stores.

To maximally enhance performance in an activity that requires prolonged (usually 2 hr or longer) strenuous exercise, during which it is not practical to ingest sufficient carbohydrate, it would be advantageous to start with high glycogen stores and a high plasma FFA concentration. However, there is no physiological way to achieve this combination. An approach that has commonly been used to artificially increase plasma FFA for research purposes is to raise plasma triglycerides by means of a fat meal, or intravenous infusion of a triglyceride emulsion, followed by injection of heparin (46-52). The heparin releases lipoprotein lipase from the capillaries, with activation of lipolysis and a large increase in plasma FFA. Use of this procedure has provided the strongest evidence that increased FFA oxidation during exercise spares both hepatic and muscle glycogen and, thus, enhances endurance (46-52). Using this approach, it has been shown in rats that raising plasma FFA slows liver and muscle glycogen utilization, delays development of hypoglycemia and markedly improves endurance (46,47). Studies in humans

in whom plasma FFA level was increased by this procedure have also shown that FFA oxidation is increased, and that utilization of muscle glycogen and blood glucose is markedly reduced (49-51).

The mechanism by which fatty acid oxidation spares carbohydrate during exercise is currently not clear. It was thought that the classical glucose-fatty acid cycle (53-55), with an increase in muscle citrate and inhibition of phosphofructokinase, and an increase in acetyl CoA with inhibition of pyruvate dehydrogenase, was responsible. Studies on exercised rats with experimentally elevated plasma FFA supported this view, as their muscle citrate concentrations were increased compared to controls (46). However, more recent studies have found no increase in citrate in muscle biopsies of people exercising with elevated plasma FFA, raising doubts regarding this mechanism (50). This is an area that requires further study. Future studies of the mechanisms involved in the sparing of carbohydrate by FFA will have to be designed to take into account the finding that increased FFA oxidation spares not only blood glucose (liver glycogen), but also muscle glycogen. It has been assumed that this decrease in the rate of muscle glycogen depletion is mediated by a reduced rate of glycogenolysis. As is discussed in detail later in this review, the two most important factors that regulate the rate of glycogen breakdown in muscle are phosphorylase activity and inorganic phosphate (Pi) concentration. In the context of what is known regarding the regulation of phosphorylase activity and Pi concentration in muscle, it seems unlikely that oxidation of fat instead of carbohydrate could result in a decrease in either of these variables. The alternative possibility that glycogen breakdown is unaffected, but is partially countered by increased glycogen synthesis should, therefore, also be considered. The increase in glycogen synthesis could result both from channeling of some of the glucose taken up by the muscles to glycogen synthesis instead of glycogenolysis, as well as from glycogen resynthesis from its breakdown products (i.e. futile cycling).

Just as increased FFA availability and oxidation spares muscle and liver glycogen, increased availability and utilization of carbohydrate decreases the contribution of FFA oxidation to energy supply during exercise (29-32). One mechanism by which carbohydrate ingestion within 90 min or so before exercise decreases FFA oxidation is by raising blood glucose level and stimulating insulin secretion (29,30,48,56-58). The resulting increase in plasma insulin level inhibits lipolysis and, thus, lowers plasma FFA concentration, while stimulating increased muscle glucose uptake. A second mechanism by which increased carbohydrate utilization decreases fat utilization is by inhibiting the uptake and oxidation of FFA by muscle mitochondria (30,31,37). This effect appears to be mediated by an increase in muscle malonyl CoA (59-61). Malonyl-CoA is a potent inhibitor of carnitine palmityl transferase 1 (CPT 1) which is the rate-limiting enzyme of the fatty acid oxidation pathway (59). Exercise results in an activation of AMP kinase in muscle (62-64). The activated AMP kinase phosphorylates and inactivates acetyl-CoA carboxylase, which is the enzyme that converts acetyl-CoA to malonyl-CoA (65,66). As a consequence, malonyl-CoA concentration declines, resulting in an increase in CPT 1 activity and an enhanced rate of fatty acid oxidation (61-64,66,67). However, when glycolysis is increased, malonyl-CoA synthesis is enhanced, resulting in a higher concentration of malonyl-CoA (i.e. smaller decrease in malonyl-CoA), greater inhibition of CPT 1, and a reduced oxidation of FFA at a given submaximal work rate (61,67). Thus, increased availability of FFA results in increased fat oxidation and less carbohydrate utilization, while increased carbohydrate availability results in increased carbohydrate utilization and decreased fat oxidation during submaximal exercise. The studies on the effects of exercise and glycolysis on malonyl CoA concentration reviewed above were done on rats, and extrapolation of these findings to humans must be considered tentative until they are confirmed in studies on people.

4. THE ROLE OF EXERCISE INTENSITY

Exercise intensity is expressed both in absolute and relative terms. Relative exercise intensity is generally expressed as a percentage of an individual's maximal oxygen uptake capacity (VO_2max). VO_2max varies over a wide range among individuals, depending on level of aerobic training, genetic makeup, age, health status and sex. As a consequence, the same relative exercise intensity is attained at very different absolute work rates in individuals who differ markedly in their VO_2max. For example, a champion cross-country skier with a VO_2max of 90 $ml \times kg^{-1} \times min^{-1}$ has to generate energy at 6-times as great a rate as an untrained 85 year old man with a VO_2max of 15 $ml \times kg^{-1} \times min^{-1}$, when both are working at 75% of VO_2max. Assuming that the fuel mixture used by these individuals is the same, the cross-country skier's muscle mitochondria would be oxidizing 6-times as much pyruvate and fatty acids as those of the 85 yr old's during the exercise, and the rates of glycogenolysis and lipolysis would, therefore, have to be similarly higher in the athlete than in the 85 yr old. As illustrated by this extreme example, the absolute work rate determines the rate at which fuel is utilized by the muscles during exercise, and therefore, plays an extremely important role in the regulation of substrate metabolism during exercise.

While the absolute work rate determines the total quantity of fuel required by the muscles during exercise, the relative exercise intensity is a major factor in determining the fuel mixture, i.e. the proportions of carbohydrate and fat, oxidized by the working muscles (27,68). During exercise performed after an overnight fast, 70-90% of the energy required at low exercise intensities in the range of ~25-30% of VO_2max is supplied by the oxidation of fat. As shown in figure 1, as relative exercise intensity is increased from ~40% to ~85% of VO_2max, there is a decrease in the percentage of the total energy requirement derived from fat oxidation and a reciprocal increase in carbohydrate oxidation. In addition to the decline in the relative contribution of fat oxidation with increasing exercise intensity, there is a decrease in the absolute amount of fat that is oxidized at higher relative

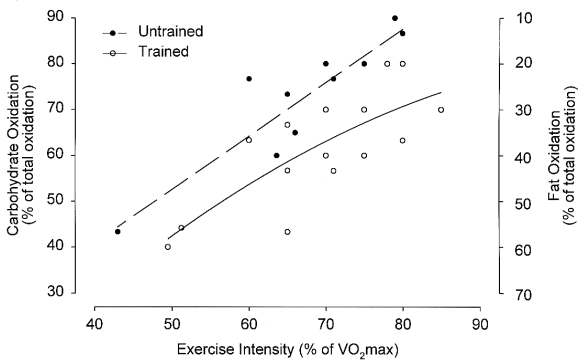

Figure 1. Carbohydrate (left axis) and fat (right axis) oxidation during submaximal exercise in untrained (closed symbols) and endurance-trained (open symbols) individuals. Taken from references (27,28,33,34,85-87).

work rates (27,37). Both plasma glucose and muscle glycogen utilization increase as exercise intensity is raised, with plasma glucose providing ~10 to 15% of total energy at all work rates and muscle glycogen providing the bulk (60% or more) of the energy required for very strenuous exercise requiring more than ~80% of VO_2max (27,68). At low exercise intensities (20-30% of VO_2max) plasma fatty acids provide nearly all of the fat that is oxidized, while at moderate and heavy intensities (50-85% of VO_2max) plasma fatty acids and muscle triglycerides provide roughly equal amounts of the fat that is oxidized (27,34,38).

The regulatory mechanisms responsible for the progressive rise with increasing exercise intensity in the proportion of total energy provided by carbohydrate oxidation are still not fully understood. Factors that play important roles include a decrease in plasma fatty acid availability due to a reduction in the amount of fatty acids released from adipose tissue (27,69), increased activation of glycogenolysis (27), and an increased recruitment of fast twitch, i.e. Type II, muscle fibers. The decrease in fatty acid release is thought to be due, at least in part, to constriction of the vascular bed in adipose tissue as a result of increased b-adrenergic stimulation (69). Direct evidence that limited availability of plasma fatty acids plays a role in the decrease in fat oxidation during high intensity exercise is provided by the finding that fat oxidation increases and muscle glycogen utilization decreases when plasma fatty acids are raised by means of infusion of triglyceride emulsion and heparin even during very intense exercise requiring 85% of VO_2max (50,52). That glycogenolysis-glycolysis has an inhibitory effect on fat oxidation has been demonstrated in a number of recent studies as discussed earlier; this effect appears to be mediated by an increase in malonyl CoA, which inhibits the enzyme responsible for transporting long chain fatty acids into the mitochondrial matrix, palmityl carnitine transferase I (59). With regard to the role of muscle fiber type, during low intensity exercise the work is performed by slow-twitch, Type I fibers which have a high capacity for fat oxidation and a low capacity for glycogenolysis-glycolysis; as the exercise intensity is increased, progressively more fast-twitch, Type II fibers, which have a high capacity for, and obtain much of their energy from, glycogenolysis-glycolysis are recruited to contract (70).

5. ROLE OF EXERCISE DURATION

During moderate intensity exercise, in the range of 55% to 75% of VO_2max, that can be maintained for 90 minutes or longer, there is a progressive decline in the proportion of energy derived from muscle glycogen and muscle triglycerides, and a progressive increase in plasma fatty acid oxidation (27). During the first 30 minutes or so of moderate intensity exercise in the fasting state, plasma fatty acids and muscle triglycerides provide roughly equal amounts of the fat that is oxidized. When the exercise is continued beyond 30 minutes, oxidation of plasma fatty acids provides progressively more of the total energy requirements, compensating for the decreased utilization not only of muscle triglycerides but also of muscle glycogen (27). This increase in plasma fatty acid oxidation during prolonged exercise is made possible by a progressive rise in plasma fatty acid concentration, and is necessitated by a progressive depletion of muscle glycogen and triglyceride stores.

6. FACTORS THAT INFLUENCE THE PROPORTIONS OF CARBOHYDRATE AND FAT UTILIZED AT THE SAME RELATIVE EXERCISE INTENSITY

Brooks has emphasized the importance of exercise intensity relative to VO_2max in determining the contributions of carbohydrate and fat oxidation to total energy utilization (71,72). He has coined the term "crossover point" for the relative exercise intensity at which the predominant fuel for oxidative metabolism shifts from fat to carbohydrate (71,72). The crossover point, which usually occurs at ~50% of VO_2max in untrained or mildly trained individuals who have been eating a usual, mixed diet, is a useful concept. It provides a reference point for evaluating the effects of various physiological and metabolic states and adaptations such as diet and exercise-training on the fuel mixture used during exercise.

Probably the most potent intervention that alters the crossover point is diet. A number of studies have shown that eating a high fat diet for prolonged periods markedly increases the contribution of fat to oxidative metabolism at the same relative exercise intensity (figure 2) (39,44,45,73-76). For example, Jansson and Kaijser (39) found that cycling at 65% of VO_2max elicited a respiratory exchange ratio (RER) of 0.92 in subjects on a high carbohydrate diet, and an RER of 0.81 in the same subjects after they had been eating a high fat diet for 7 days. An R of 0.81 indicates that carbohydrate oxidation is providing 34% of the total energy; thus these subjects were still well below the crossover point when exercising at 65% of VO_2max on a high fat diet. An even more marked effect is seen with prolonged adaptation to a high fat diet. In a study in which subjects ate a high fat diet for 49 days, Helge, *et al.* (73) found that they were far below the crossover point, with an RER of 0.82, indicating that only 38% of energy came from carbohydrate oxidation, during

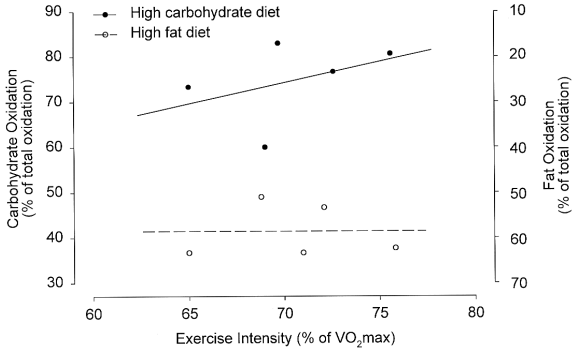

Figure 2. Effects of diets high in carbohydrate (closed symbols) or fat (open symbols) on carbohydrate (left axis) and fat (right axis) oxidation during submaximal exercise. Taken from references (39,44,45,73,75).

exercise at 70% of VO_2max. It is interesting that although relatively short periods on a high fat diet decreases exercise endurance (14,44,45) by lowering muscle glycogen, more prolonged adaptation to a high fat diet may result in no decrease in, or actually enhance, endurance performance in some individuals at exercise intensities in the 60-70% of VO_2max range (15,74-78). The mechanism responsible for the improvement in endurance appears to be a high fat diet-induced increase in mitochondrial enzymes, particularly those involved in fatty acid oxidation, in skeletal muscle (77,79).

Another factor that can play an important role in determining the crossover point is a decrease in muscle glycogen concentration during exercise. Costill *et al* (80) studied the effect of running 16.1 km at 80% of VO_2max on three successive days on muscle glycogen and RER in 5 well trained men. Each bout of exercise resulted in a marked decrease in muscle glycogen, and glycogen repletion from one day to the next was minimal. As a result, initial and final muscle glycogen levels were lower during the run on day 2 than on day 1, and lower on day 3 than on day 2. During the first 16.1 km run at 80% of VO_2max, 87% of the energy was provided by carbohydrate oxidation, during the second run 66% of the energy came from carbohydrate, and during the third trial carbohydrate oxidation provided only 57% of the energy (80).

A number of other studies have shown that lowering glycogen stores by means of exercise followed by a low carbohydrate diet results in increased oxidation of fat during exercise (45,81,82). This increase in fat oxidation is mediated in part by increases in the level of plasma fatty acids as the result of a reduction in plasma insulin and elevation of catecholamine levels (45). It was not clear from these studies whether the metabolic effects of low body carbohydrate stores during exercise are mediated by liver or muscle glycogen depletion or by a decreased blood glucose level. In an ingenious series of experiments, in which they maintained euglycemia or raised blood glucose levels and varied plasma insulin concentration, Noake's group was able to demonstrate metabolic and hormonal effects of carbohydrate depletion that are the result of low muscle glycogen content (32,83). They found that a low muscle glycogen content results in increases in plasma fatty acids and norepinephrine, increased fat oxidation and decreased muscle glycogen utilization during exercise. How the effects of low muscle glycogen on plasma fatty acids, insulin and norepinephrine are mediated is a fascinating, still unanswered question. During exercise at 70% of VO_2max, the subjects with normal muscle glycogen were above the crossover point for the entire 145 minutes exercise period, while the subjects with low muscle glycogen were below the crossover point for the last 80 minutes of exercise at 70% of VO_2max (32).

The crossover point can also be raised by adaptation to endurance exercise training (figure 2). The training must be intense and the adaptive response large, as moderate training that results in a small increase in aerobic power does not affect the crossover point (84). In a study in which 8 men underwent 12 wk of intense training that raised their VO_2max 26%, RER at the end of 10 minutes of exercise that elicited 60% of VO_2max was 0.93 before (76% of energy from carbohydrate) and 0.89 (62% of energy from carbohydrate) after training (85). A group of highly trained endurance athletes were studied at the same time. At an exercise intensity that required 80% of VO_2max, the athletes RER was 0.89 (62% of energy from carbohydrate; the same as that of the subjects after training exercising at 60% of VO_2max) compared to an RER of 0.96 (86% of energy from carbohydrate) in the untrained subjects (85). In a study comparing highly trained with untrained individuals, in which rates of glucose appearance and disappearance were measured, the trained individuals obtained more of their energy from fat and less from carbohydrate oxidation during exercise requiring 80% of VO_2max (86). In another study in which young and older trained and untrained men were compared at an exercise intensity of 70% of VO_2max, the trained groups obtained ~55% of their energy from carbohydrate oxidation, while in the untrained young men ~76% of the energy utilized was provided by carbohydrate oxidation (87).

7. REGULATION OF GLUCOSE AND GLYCOGEN UTILIZATION IN MUSCLE DURING EXERCISE AND THE EFFECT OF TRAINING

Transport of glucose through the sarcolemma is the primary rate-limiting step for glucose metabolism in striated muscle. This process has been reviewed in detail (3-5). Glucose transport into the muscle cell occurs by means of a passive transport mechanism that does not use ATP. It is a saturable process that is mediated by glucose transporter proteins, of which two isoforms are expressed in skeletal muscle (88-90). The less abundant GLUT1 isoform is thought to reside primarily in the sarcolemma and contributes to basal glucose transport. The GLUT4 isoform is the major glucose transporter in skeletal muscle. In the basal state, most of the GLUT4 are components of intracellular vesicles. Glucose transport into muscle is stimulated by at least two separate pathways, one of which is activated by insulin, the other by muscle contractions. The increases in glucose transport induced by contractions and by insulin are mediated by a translocation of the GLUT4 vesicles from intracellular sites to the sarcolemma

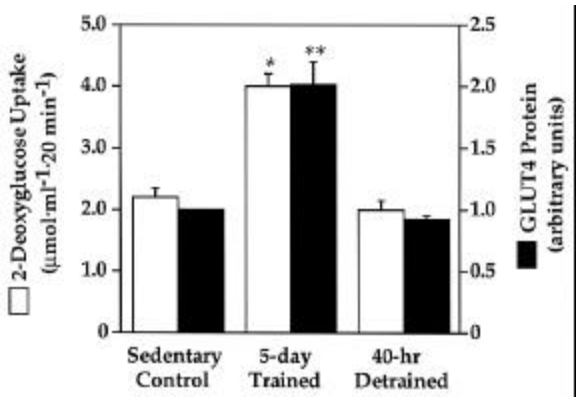

Figure 3. Effect of 5 days of exercise training and 40 hr of rest, posttraining on maximally insulin-stimulated glucose transport activity (open bars) and GLUT4 protein content (solid bars) in rat epitrochlearis muscles. Values are the means ± SE for 6-12 rats/group. *P<0.01, **P<0.001 versus sedentary control and 40-hr detrained. From reference (138).

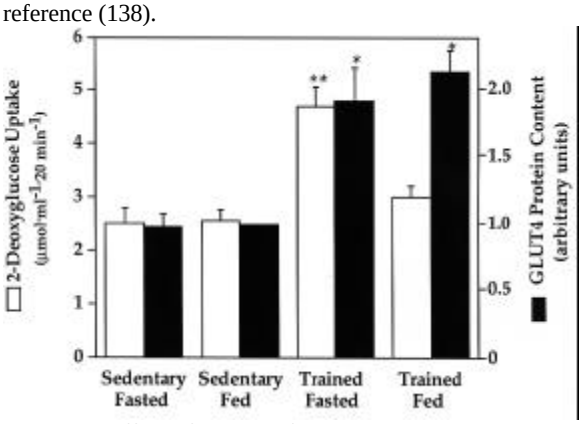

Figure 4. Effect of 2 days of swim training on maximally insulin-stimulated glucose transport activity and GLUT4 protein concentration in rat epitrochlearis muscles. Following the last bout of exercise, animals were either fasted or fed standard rodent chow ad libitum for ~18 hr prior to assay of 2-deoxyglucose uptake and GLUT4 expression. Glycogen concentrations in muscles of the four groups at the time of the assay were as follows: control fasted, 13 mmol/g; trained fed, 81 mmol/g muscle wet weight; control fed, 28 mmol/g; trained fasted, 10 mmol/g; trained fed, 81 mmol/g. *P<0.001 versus sedentary groups. **P<0.001 versus all other groups. From reference (140).

with fusion of the vesicles with, and incorporation of the GLUT4 protein into, the sarcolemma (88,91-95). Most of the GLUT4 containing vesicles are translocated to the transverse tubules (96,97). These invaginations of the sarcolemma extend deep into the muscle and are adjacent to the sarcoplasmic reticulum. This arrangement makes possible the transport of glucose directly into the interior of the muscle cells where much of the ATP utilized by the myofibrils during contractile activity is generated via aerobic glycolysis and oxidation of the pyruvate that is formed. The transverse tubules are also adjacent to the sarcoplasmic reticulum (SR), which is the region where much of the glycogen resynthesis after exercise occurs in the glycogen-protein complexes associated with the SR. The increases in GLUT4 at the cell surface and in glucose transport induced by maximally effective contraction and insulin stimuli are additive, providing evidence that two separate pathways are involved (93,98-102). The finding that it is possible to inhibit insulin-stimulated, but not contraction-stimulated, glucose transport with the phosphatidylinositol (PI) 3-kinase inhibitor wortmanin provides further evidence for two pathways (93,103,104).

Exercise has three separate, well documented effects on muscle glucose transport (3). The first of these is an insulin-independent stimulation of glucose transport that occurs during normal exercise such as running or swimming (105-108), as well as during electrical stimulation of muscle contractions (109,110). This effect persists sufficiently long to be measurable immediately after contractile activity, but usually wears off completely within 60 minutes or so, in muscles incubated *in vitro* (111,112). The mechanisms by which muscle contractile activity brings about a movement of GLUT4 into the sarcolemma have not yet been elucidated. However, considerable evidence suggests that the first step in the pathway by which exercise stimulates glucose transport is the increase in cytosolic Ca^{2+} that occurs as a result of release of Ca^{2+} from the sarcoplasmic reticulum during excitation-contraction coupling (113-116).

As the acute, insulin-independent effect of exercise wears off, it is replaced by a large increase in the sensitivity of the glucose transport process to insulin (112,117-121). Insulin sensitivity is defined in terms of the insulin concentration required to cause one-half of its maximal effect. The term insulin responsiveness refers to the increase in glucose transport caused by a maximally effective insulin stimulus. The increase in insulin sensitivity following exercise reverses as muscle glycogen supercompensation occurs (120). Feeding a high-carbohydrate diet speeds the reversal of the increase in muscle insulin sensitivity after exercise, while fasting or feeding a carbohydrate-free diet to prevent glycogen supercompensation results in persistence of the increase in insulin sensitivity for days (120). Glycogen supercompensation is the term used to describe the increase in muscle glycogen concentration above the usual, fed level in response to carbohydrate feeding following glycogen depleting exercise. The mechanism responsible for the increase in muscle insulin sensitivity after exercise has not yet been elucidated. However, it is known that a serum factor is required (122), that sensitivity of glucose transport to contractions and hypoxia is also increased (121), and that translocation of more GLUT4 into the sarcolemma in response to a given submaximal insulin stimulus is responsible for the increased glucose transport activity (123).

Another effect of exercise that influences muscle glucose transport is an adaptive increase in GLUT4 protein (figures 3,4). Endurance exercise-training induces a number of major adaptations in skeletal muscle. These include an increase in muscle mitochondria with an enhancement of the capacity to oxidize carbohydrate and

fatty acids (124-126). The half-time of this adaptive increase in mitochondria is ~6 days (127-129). Increases in GLUT4 protein and hexokinase are components of this adaptive response (130-136). The GLUT4 protein has a very short half-life, and, as a result, the increase in GLUT4 occurs even more rapidly than the increases in most of the mitochondrial enzymes (137). In studies on rats, it was found that the adaptive increase in GLUT4 protein in response to a large exercise stimulus plateaus within ~48 hours (137). The adaptive increase in GLUT4 also reverses very rapidly in rats (within 40 hr), after exercise is stopped (figure 3) (138).

In the absence of conditions, such as visceral obesity, that cause resistance of glucose transport to the actions of insulin and contractile activity, the GLUT4 content of a muscle determines its maximally stimulated glucose transport capacity (figure 3) (101,139). As a consequence, the adaptive increase in muscle GLUT4 induced by exercise is reflected in increases in maximally insulin-stimulated, maximally contraction-stimulated, and maximally contraction-plus insulin-stimulated glucose transport (131,137,138). However, if glycogen supercompensation is allowed to occur after exercise the effect of the increase in GLUT4 induced by training becomes masked so that, despite the increase in GLUT4, the effect of a maximal insulin stimulus on glucose transport is no greater than in control, untrained muscle (figure 4) (140).

The adaptations induced by endurance exercise training are associated with a marked sparing of carbohydrate during exercise, with a slower utilization of plasma glucose, liver glycogen and muscle glycogen during sustained exercise of the same intensity after, as compared to before, training (9,33,141-144). It seems surprising, in view of the increases in muscle insulin sensitivity and GLUT4 content in individuals who exercise regularly, that trained individuals have slower rates of glucose uptake and utilization during exercise of the same absolute exercise intensity than they did in the untrained state (9,84). This apparent discrepancy makes good sense from a teleological viewpoint, because the more rapidly glucose and glycogen are used during exercise, the sooner the individual is forced to stop exercising by either the development of hypoglycemia or depletion of muscle glycogen. Thus the slower utilization of blood glucose and muscle glycogen during exercise in the trained state are among the important mechanisms by which exercise training enhances endurance. However the biological mechanism responsible for the slower utilization of glucose in the face of increases in muscle insulin sensitivity and GLUT4 content has not yet been established. One possible mechanism that may partially explain the plasma glucose sparing effect of training could be that the GLUT4 vesicles in trained muscle are more resistant to translocation to the cell surface by contractile activity (145).

While it is well established that glucose utilization during exercise of the same absolute intensity is slower in the trained state, the effect of exercise training on the rate of plasma glucose utilization during exercise of the same relative intensity, i.e. at the same percentage of VO_2max, is less clear cut. In one study, in which the same individuals were tested before and after 10 weeks of endurance exercise training, it was found that the rate of blood glucose utilization during a standardized exercise bout of the same relative intensity was the same before and after training (84). Because the same relative exercise intensity following adaptation to endurance training requires an increased rate of energy expenditure and involves a larger muscle mass, the proportion of energy derived from carbohydrate was decreased while the proportion provided by fat oxidation was increased in this study. In another study in which untrained individuals were compared to trained athletes, a situation in which the difference in level of training was much more marked, it was found that the same relative exercise intensity resulted in a slower rate of blood glucose utilization in the trained individuals (86). In any case, it is well established that training results in a proportionally lower reliance on carbohydrate utilization and a greater reliance on fatty acid oxidation for generation of the energy required during prolonged steady state exercise.

7.1 Regulation of Glycogenolysis

It has been known since the late 1960's that muscle glycogen is required for strenuous exercise. When muscle glycogen stores are depleted muscle fatigue develops and vigorous exercise can no longer be continued (44,146-148). It is still not clear why muscle glycogen is essential for strenuous exercise when other substrates including fatty acids and blood glucose are still available. This is a question that requires further investigation. On the other hand, our understanding of how glycogenolysis is geared to work rate and the mechanism by which endurance exercise training spares muscle glycogen have been largely elucidated in recent years. During vigorous exercise, or electrical stimulation of muscles to contract in situ, at a rate at which a steady state can be maintained, there is a large initial burst of glycogenolysis followed by a marked slowing of the rate of glycogen breakdown (149-152). The increase in lactate that results from the initial burst of glycogenolysis is followed by a decrease in muscle and blood lactate levels, despite continued muscle contractile activity, as a result of the marked slowing of glycogenolysis (151-154).

The initial burst of glycogenolysis results from activation of phosphorylase by the increase in cytosolic calcium that occurs during excitation contraction coupling. Glycogen phosphorylase, which catalyzes glycogen breakdown, exists in two molecular forms. Phosphorylase b, which is inactive under the conditions usually found in resting muscle cells, is converted to the (or its') active a form by the enzyme phosphorylase kinase which requires calcium for activity (155-158). Phosphorylase kinase also exists in a dephosphorylated, less active or b form that is converted to the more active a form by protein kinase A, which is activated by the increase in cyclic AMP induced by increased catecholamine levels (155-158).

Phosphorylase kinase a can activate phosphorylase at the low intracellular Ca^{2+} concentrations

that are found in the cytoplasm of resting muscle cells; in contrast, phosphorylase kinase b is inactive in resting muscle, but becomes active at the cytosolic calcium levels that are attained when muscles are activated to contract (155-158). The classical, textbook picture of the regulation of glycogenolysis in skeletal muscle was based on the concept that glycogenolysis does not occur in resting muscle because phosphorylase is essentially completely in the inactive b form (156,157,159,160). However, it has become clear during the last 20-30 years that approximately 10% of phosphorylase is in the active a form under physiological conditions (161-163). The presence of phosphorylase a in resting muscle was initially attributed to a preparation artifact as a result of contraction of the muscle during freezing or homogenization. However, the methodology has improved to the point where muscles can be clamp frozen almost instantaneously, and it has become evident that there is always a considerable amount of phosphorylase in the active form in resting muscle (150,161-163).

According to the classical concept, it was though that glycogen breakdown is geared to muscle contraction by the transient increases in calcium concentration in the cytoplasm during each contraction. It was thought that the rate of glycogenolysis is determined by the frequency of muscle contraction, with release of Ca^{2+} from the SR during each excitation-contraction coupling resulting in conversion of inactive phosphorylase b to active phosphorylase a and a burst of glycogenolysis (155,157,159,164-166). The studies on which this original concept of glycogenolysis regulation was based were done either on purified enzymes, on glycogen-enzyme particles, or on muscles that were subjected to brief tetanic stimulation. More recently, during studies involving longer periods of stimulation, it was found that phosphorylase activation reverses within a few minutes during continued contractile activity (150,167-170), and that the reversal occurs despite sustained contractile activity in the absence of fatigue (150). If phosphorylase activation did not reverse after a short time, resulting in a slowing of glycogenolysis, prolonged vigorous exercise would be impossible. Activation of phosphorylase by the calcium mechanism results in a marked overshoot of glycogen breakdown relative to the need for energy, and it would result in rapid depletion of glycogen, massive accumulation of lactate and rapid development of fatigue if it persisted.

It has now become apparent that the initial Ca^{2+}-mediated, massive burst of glycogenolysis shuts off within a few minutes after making a large supply of pyruvate available to the mitochondria. The proportion of phosphorylase in the a form then drops back to the level found in resting muscle, i.e. approximately 10% (150,168-170). Most of the phosphorylase in muscle is bound to a glycogen-enzyme-sarcoplasmic reticulum complex that also contains phosphorylase kinase (165,171). Although the mechanism by which phosphorylase activation reverses during continuous exercise is not fully understood, it seems likely that the reversal is at least in part due to release of phosphorylase from the glycogen particle as the glycogen breaks down, thus uncoupling phosphorylase kinase and the calcium activating mechanism from phosphorylase. This mechanism may explain why the activation of phosphorylase both by contractile activity and by epinephrine is severely inhibited in glycogen depleted muscles following exercise (172)

It is well documented that prolonged strenuous exercise to the point of exhaustion can result in almost complete glycogen depletion (44). This raises the question: if the calcium mechanism for activating phosphorylase becomes inactivated soon after the onset of exercise, how does muscle glycogen depletion continue to occur? One possible explanation that has been suggested is that there may be a reactivation of phosphorylase during continued exercise via the beta adrenergic stimulation-cAMP mechanism (169,173). Arguing against this possibility is the lack of evidence that a reactivation of phosphorylase occurs during continuous exercise and the finding that, after the initial burst of glycogenolysis, the rate of glycogen breakdown is rather closely geared to the energy requirement of the working muscles.

In this context, a new explanation for the continuous breakdown of glycogen during prolonged, strenuous exercise and the gearing of glycogenolysis to work rate has evolved. It is based on two concepts that have gradually won acceptance. One is that, contrary to the classical concept that essentially all of the phosphorylase in resting muscle is in the b form, a considerable proportion, in the range of 8-15%, of muscle phosphorylase is in the a form in resting muscle *in vivo* (161-163). The other is that it is the concentration of free inorganic phosphate (Pi), not phosphorylase activity, that limits glycogenolysis under most conditions, and this is why rapid glycogen breakdown does not occur in resting muscle despite the presence of considerable phosphorylase activity (161,163,170).

Direct support for this concept was provided by a study on rat epitrochlearis muscles incubated *in vitro* in which cytosolic Pi was raised by means of hypoxia, and phosphorylase was activated with epinephrine (163). Over an 80 minutes period, hypoxia resulted in a progressive ~70% decrease in epitrochlearis muscle glycogen concentration despite no increase in % phosphorylase a. Inorganic phosphate concentration increased rapidly in response to the hypoxia, roughly mirroring the decline in phosphocreatine concentration. Incubation of oxygenated muscles with a high concentration of epinephrine for 20 minutes resulted in a large increase % phosphorylase a. Despite the activation of phosphorylase, there was only a small decrease in muscle glycogen (2.5 mmol glucose/g) over a 20 minute period (figure 5). No decrease in glycogen occurred in well oxygenated control muscles. In contrast, the muscles incubated under hypoxic conditions, in which there was no activation of phosphorylase, showed a 12 mmol glucose/g decrease in muscle glycogen over the 20 minute period (figure 5).

The very small glycogen breakdown caused by a more than six fold increase in phosphorylase activity in response to epinephrine is explained by the fact that Pi levels were unchanged and therefore limited the ability of

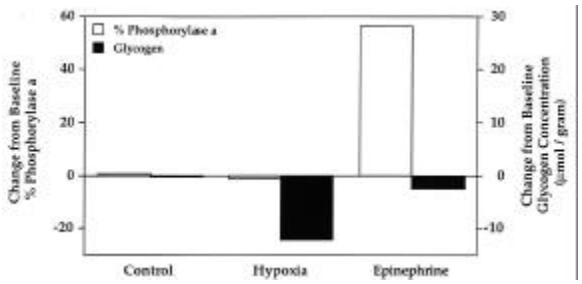

Figure 5. Changes in phosphorylase a and glycogen concentration in rat epitrochlearis muscles elicited by a 20 min *in vitro* incubation in oxygenated Krebs Henseleit buffer (KHB) (control), KHB gassed with 95% N_2-5% CO_2 (hypoxia), or oxygenated KHB containing 0.1 mM epinephrine. From reference (163).

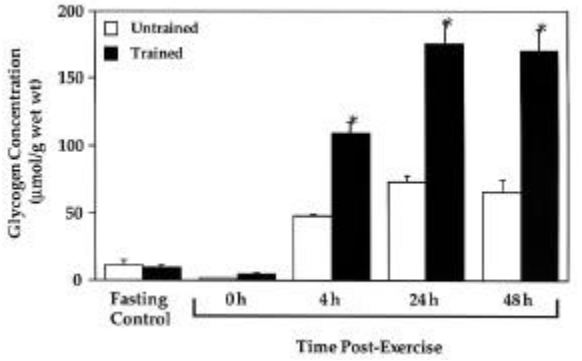

Figure 6. Time course of epitrochlearis muscle glycogen accumulation in trained (solid bars) and untrained (open bars) rats after a glycogen-depleting bout of exercise. After an overnight fast, animals performed a bout of swimming exercise, then were allowed to recover with free access to standard rodent chow and 5% sucrose in their drinking water. Muscles were harvested at the indicated times. *P<0.001 versus untrained. From reference (186).

phosphorylase to catalyze the reaction: Glycogen + Pi ® Glucose 6-P. Inhibition of phosphorylase b with 2-deoxyglucose-6-phosphate had a negligible effect on the stimulation of glycogenolysis by hypoxia, providing evidence that phosphorylase b activation by AMP was not playing a significant role (163). These findings show that the basal level of phosphorylase a activity present in non-stimulated muscle can mediate a moderately rapid rate of glycogenolysis in response to an increase in inorganic phosphate. Since the magnitude of the increase in Pi during muscle contractile activity is a function of the work rate, this mechanism explains how glycogenolysis is geared to work rate after the % phosphorylase a returns to the basal level in contracting muscle.

One of the most important physiological effects of the rapid increase in muscle mitochondria in response to endurance exercise training is the sparing of muscle glycogen during submaximal exercise (143,144,152). This effect is evident both as a smaller initial burst of glycogenolysis and a slower subsequent rate of glycogen utilization during prolonged exercise. In studies on rat skeletal muscles stimulated to contract *in situ*, it was found that the effect of training on the initial, calcium-induced burst of glycogenolysis is markedly blunted in endurance exercise trained muscle (151,153) even though there is no difference in the extent of phosphorylase activation between trained and untrained muscles subjected to the same stimulation protocol (153). This glycogen sparing effect of the adaptive increase in mitochondria in trained muscle is mediated by a smaller decrease in creatine phosphate and, therefore, a smaller increase in Pi in response to the same work rate (151,153,174). Thus, the steady state concentration of inorganic phosphate attained in muscles during continued contractile activity is lower in the trained muscles (151,153,174). It, therefore, seems probable that both the smaller initial burst of glycogenolysis and the slower rate of glycogen breakdown during continuous contractile activity is explained by the lower level of inorganic phosphate attained in trained muscles.

7.2 The Muscle Glycogen Supercompensation Phenomenon

The term "glycogen supercompensation" refers to the large increase in muscle glycogen concentration, far above the levels found in the well-fed, sedentary state, that occurs in response to carbohydrate feeding following a glycogen-depleting exercise bout (44,175-177). Glycogen supercompensation is limited to the muscles in which glycogen was depleted by the exercise. This was clearly shown by Bergström and Hultman (176) who first discovered the supercompensation phenomenon in a study in which they used themselves as subjects.

Glycogen synthase D, the inactive form of the enzyme, is converted to the active form, glycogen synthase I, by the action of glycogen synthase phosphatase (178,179). Both of these enzymes are bound to glycogen, and when glycogen is broken down during exercise they are released into the cytosol. This makes the inactive glycogen synthase D accessible to glycogen synthase phosphatase, which converts it to the active form, glycogen synthase I (180,181).

There has been much interest in the concept that glycogen synthase activity limits and determines the rate of muscle glycogen synthesis (177,182-184). With regard to the synthesis of muscle glycogen after glycogen-depleting exercise, it is now well documented that the increase in the proportion of glycogen synthase in the active I form is only involved in the early, rapid phase of glycogen repletion. It does not play a role in glycogen supercompensation. The evidence for this is that the activation of glycogen synthase reverses, with a decline in the proportion of glycogen synthase in the I form to a low level, when muscle glycogen concentration attains the usual, fed sedentary level, i.e. before glycogen supercompensation begins (185,186). Thus, glycogen supercompensation occurs despite low glycogen synthase activity.

The factor that regulates the rate and extent of muscle glycogen accumulation appears to be the rate of glucose transport into muscle. The factors that determine the rate of glucose transport are the number of GLUT4 at the cell surface and the concentration of glucose in the

interstitial space. One line of evidence that the rate glucose entry into muscle exerts the primary control on glycogen synthesis comes from studies on transgenic mice that overexpress the GLUT1 glucose transporter in their muscles (187). Basal glucose transport is increased approximately 7-fold in muscles of GLUT1 transgenic mice. This results in a massive accumulation of glycogen in their muscles, to values ten-fold higher than those found in muscles of fed normal wild-type mice despite a 50% reduction in glycogen synthase I activity (187).

Further evidence is provided by a more physiological model, rats that have adapted to exercise with an increase in the GLUT4 content of their muscles (137,186). This adaptation occurs very rapidly and also reverses quickly (137,138). Muscles of rats that have adapted to exercise with an increase in GLUT4 have increased rates of glucose uptake and glycogen synthesis than muscles of untrained animals when the muscles are incubated *in vitro* with glucose and insulin despite no difference in glycogen synthase I activity (137). Furthermore, exercise-trained rats and humans with increased numbers of GLUT4 in their muscles have markedly greater rates of muscle glycogen resynthesis and attain higher levels of muscle glycogen supercompensation than untrained controls (186,188) (Figure 6).

What physiological advantage does this adaptation confer. There is extensive evidence that starting a prolonged bout of exercise requiring ~75% of VO_2max with a markedly supercompensated muscle glycogen store postpones fatigue and improves performance, because strenuous exercise can not be continued once muscle glycogen is depleted (9,44,148,189-192). However, this beneficial effect of marked glycogen supercompensation is limited to moderately strenuous exercise lasting in the range of ~90 to 180 minutes (see (190) for review). For shorter periods of moderately strenuous to strenuous exercise (i.e. ~60 to 100% of VO_2max), starting with glycogen supercompensated muscles provides no benefit (190), while more prolonged moderately strenuous exercise requires carbohydrate ingestion during the exercise. In this context, it seems likely that the major beneficial effect of the adaptive increase in GLUT4, that provides the survival benefit for which it was selected, is the increased ability to rapidly resynthesize glycogen to replenish depleted muscle glycogen stores. As reviewed earlier, vigorous exercise becomes impossible once muscle glycogen becomes depleted, making fight or flight impossible under life-threatening conditions. The rapid adaptive increase in muscle GLUT4 in response to exercise makes possible more rapid muscle glycogen repletion when carbohydrate is eaten during brief rest periods, or even on-the-run (193). This would provide a survival advantage in situations in which a sustained increase in physical activity becomes necessary as, for example, when an animal's territory is invaded by predators.

8. CONCLUSION

Over the past decade, there has been an impressive increase in what is known regarding the regulation of carbohydrate and fat metabolism. A major factor contributing to progress in this area has been the application of new technologies and methodologies. However, a number of fundamental questions remain unanswered despite many years of intense investigation. The more intractable of this include: a) What is the mechanism by which fatty acids inhibit glucose uptake and oxidation by muscle?; b) How is hepatic glucose production geared so accurately to glucose utilization to maintain blood glucose level constant during prolonged mild to moderate exercise? c) What are the factors responsible for the lower rate of muscle glucose uptake in the trained than in the untrained state despite the increase in GLUT4?and d) Why is muscle glycogen necessary for vigorous exercise? Trying to answer these and the many other unanswered questions should keep those of us interested in the regulation of carbohydrate and fat metabolism during exercise fully employed for the remainder of our research careers.

9. ACKNOWLEDGMENTS

The authors' research is supported by NIH Research grants DK18986 and AG00425, Institutional National Research Service Award AG00078, General Clinical Research Center Grant 5 MO1RR-00036, and Diabetes Research and Training Center Grant AM20579. The expert assistance of Victoria Reckamp in the preparation of this manuscript is gratefully acknowledged.

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Key Words: Energy Requirement, Fatty Acids, Glycogen, Liver, Muscle, Triglycerides

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