CHAPTER 17

Metabolic Systems: Substrate Utilization

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This chapter traces advances made during the past century in our understanding of the effects of acute and chronic exercise on substrate metabolism. The chapter emphasizes the integrative physiology of substrate metabolism in exercising humans, although selected studies using animal models are also considered. Because chapter 1 did not cover the topic of substrate utilization in great detail, the following section is presented first.

Substrate Utilization During Exercise: Understanding Before 1910

As discussed by previous authors (4, 9, 19), many of the salient features of the metabolic response to exercise were initially described well before 1910. For example, in 1807 Berzelius reported that lactate concentrations were higher in the fully functioning muscles of hunted deer than in the partially paralyzed muscles (15). In 1845, von Helmholtz observed a decrease in alcohol-soluble substances and an increase in water-soluble substances in muscle with activity, a finding consistent with the breakdown of glycogen to form lactate and other glycolytic intermediates (179). In 1859, du Bois-Reymond determined that muscle contractions were associated with an increase in H^+ concentration, which he concluded was due to the production of lactic acid from glycogen (62). In 1871 Weiss was the first to directly demonstrate that muscle glycogen was utilized during contractile activity (183), and in 1887 Chauveau and Kauffman found in arteriovenous balance experiments that glucose uptake by muscle increased several-fold during exercise (27). Before this, in 1862 Smith reported that treadmill exercise did not increase the daily loss of nitrogen in the urine of prisoners consuming a constant diet (168), indicating that, contrary to what was previously thought, protein was not an important energy source during exercise. That exercise did not significantly increase protein oxidation was confirmed a few years later in experiments by Fick and Wislicenus (70) and von Pettenkofer and Voit (180).

According to Nathan Zuntz (1847-1920) writing in 1911 (191), findings such as these led to the belief that carbohydrate was the sole source of energy used by exercising muscle. However, based on measurements in his own laboratory of the respiratory exchange ratio (RER) in exercising horses and humans starting in the late 1880s (reviewed in 191), Zuntz believed that both fat and carbohydrate could be oxidized by muscle during exercise and that the relative contribution from each depended on the intensity and duration of the exercise as well as the availability of bodily carbohydrate stores. Zuntz also recognized that, in addition to muscle glycogen, liver glycogen was an important source of fuel for contracting muscle and that liver glycogenolysis served to maintain blood glucose concentration during moderate exercise and elevate it during strenuous exercise. Finally, on the basis of his own experiments as well as those of others, Zuntz understood that the oxidation of carbohydrate yielded only slightly more energy per unit of O_2 consumed than the oxidation of fat did. Indeed, as emphasized by Barnard and Holloszy (9), perhaps the only point on which Zuntz's understanding of substrate metabolism during exercise differed substantially from that of the present day was his mistaken belief that the production of lactate by exercising muscle was due to hypoxia.

Substrate Utilization During Exercise: Advances From 1910 to World War II

Despite Zuntz's quite modern view as expressed in his 1911 review, it took approximately another 30 yr of research before a widely agreed-upon (albeit flawed, as shall be discussed) picture of substrate utilization dur-

ing exercise fully emerged. During these years, investigation in this area focused primarily on clarifying two important issues:

- What substrate(s) are in fact oxidized during exercise?
- What is the immediate source of energy fueling muscle contraction?

These two interrelated questions remained unsettled largely because of the influence of two individuals—J.B. Auguste Chauveau (1837-1917) and especially Archibald V. Hill (1886-1977) of England—and because basic research in biochemistry was not at the level of physiological measurements.

On the basis of experiments in which RER was measured during intense exercise leading to exhaustion in a little more than 1 h, Chauveau held that carbohydrate was the only fuel directly oxidized during exercise and that fat could be used only if it was first converted into carbohydrate, a process that he estimated would reduce the net energy yield by approximately 30% (27). Although this 1896 hypothesis was inconsistent with the results of Nathan Zuntz's experiments performed at around the same time, Chauveau's intellectual influence was such that the first question listed previously remained a point of controversy for quite some time.

The concept that carbohydrate was the sole fuel used by exercising muscle was further reinforced by the publication in 1907 of Walter M. Fletcher (1873-1910) and Frederick G. Hopkins' (1861-1947) now-classic studies definitively demonstrating the production of lactate by contracting muscle (72). This was followed closely thereafter by the publication of a series of papers between 1910 and 1914 by Fletcher's student Hill, who demonstrated that muscle contraction was accompanied by the liberation of the same amount of heat regardless of whether O_2 was present (94-96). If O_2 was present, however, additional heat was released during the recovery process. These observations led Hill, as well as Fletcher and Hopkins, to believe that formation of lactate was directly responsible for muscle contraction and that O_2 was used to rebuild lactate into its parent molecule during recovery. This parent molecule was originally believed to be Hermann's inogen (92) but was later accepted to be glycogen after experiments by Parnas and Wagner in 1914 (148) and Otto F. Meyerhof (1884-1951) in 1920 (137).

The controversy over whether carbohydrate alone or both fats and carbohydrate were oxidized during exercise led Francis G. Benedict (1870-1957) and Edward P. Cathcart (1877-1954) to readdress this issue in 1913 (11). Using a professional cyclist as their primary research subject, Benedict and Cathcart conducted an extensive series of experiments in which they quantified the rates of O_2 uptake ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) as well as heart rate, rate of respiration, and, in some experiments, rectal temperature before, during, and after exercise performed at various intensities on a carefully calibrated cycle ergometer. These experiments clearly demonstrated that although RER generally increased with the transition from rest to exercise, it typically remained well below unity even at a $\dot{V}O_2$ as high as 45 $ml \cdot min^{-1} \cdot kg^{-1}$. Furthermore, they revealed that both gross and net efficiency were essentially constant during exercise performed at a somewhat lower intensity despite a large difference in RER (i.e., 0.77 vs. 0.88-0.90) induced by the subject's preceding diet. This led Benedict and Cathcart to at least tentatively reject Chauveau's hypothesis, concluding that "although the evidence in all of (our) experiments . . . is strikingly in favor of the view that during periods of muscular work there is an increased draft upon carbohydrate material in the body, the data by no means indicate that muscular work is performed exclusively by the combustion of carbohydrate" (11, p. 94) and that "the fact that the experiments with the diet poor in carbohydrates showed not the slightest indication of an increase in the energy output per unit of work is strongly suggestive of the absence of the transformation during work of fat to glycogen with a consequent liberation of unavailable heat" (11, p. 146). That Benedict and Cathcart did not dismiss Chauveau's idea completely despite their completely contradictory findings speaks to the sway Chauveau held over the field at the time.

Benedict and Cathcart's experiments were essentially replicated in 1920 by August Krogh (1874-1949) and Johannes Lindhard (1870-1947), who, distrustful of the intermittent collection of expired air during exercise using a mouthpiece and closed-circuit system as employed by Benedict and Cathcart, used an elaborately constructed and extensively validated Jacquet-type respiration chamber that completely enclosed the subject and the cycle ergometer to continuously measure respiratory gas exchange using the open-circuit approach (figure 17.1) (118). Regardless of this difference in methodology, however, Krogh and Lindhard's results were quite similar to those obtained by Benedict and Cathcart. RER tended to be higher during exercise than at rest (especially after several days of a high-carbohydrate diet) but still remained well below unity. Also, although the subjects' energy expenditure appeared to be slightly greater when the rate of fat oxidation was higher, the difference was only 11%—much smaller than the 30% suggested by Chauveau or the minimum of 24% recalculated by Zuntz.

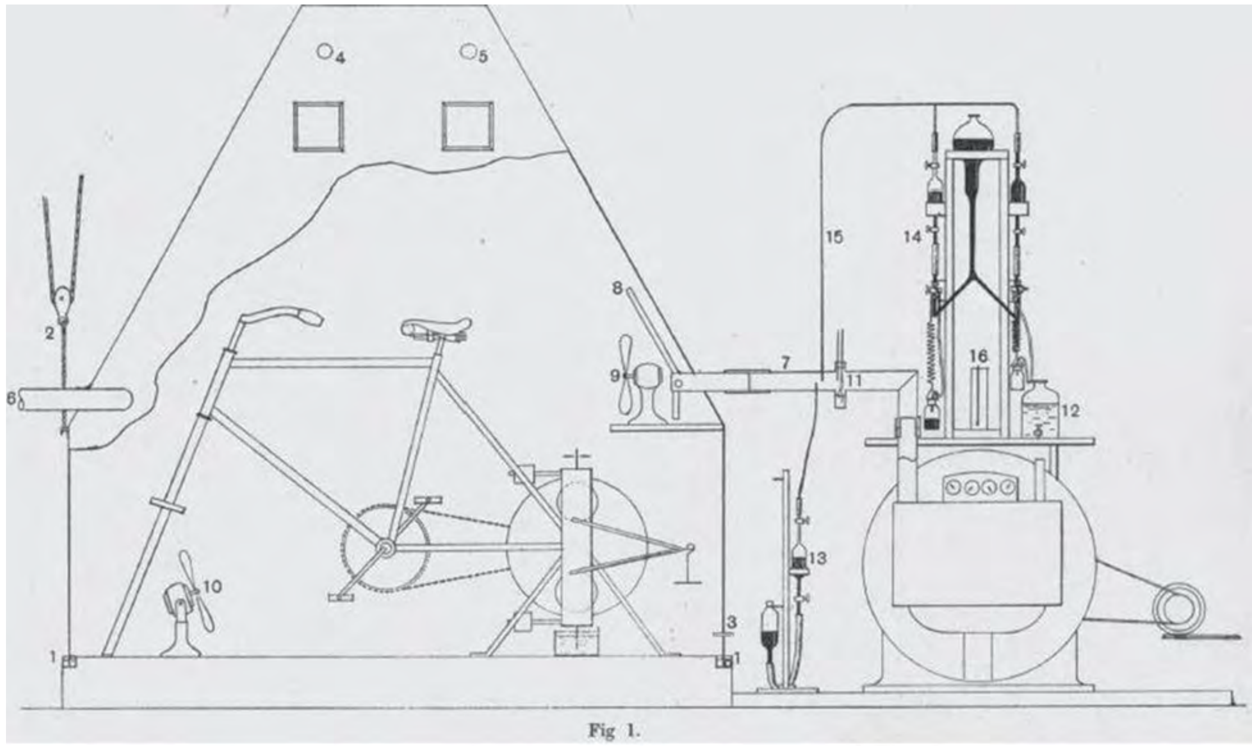

Figure 17.1 The cycle ergometer and Jacquet-type respiration chamber used by Krogh and Lindhard.
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Despite such data, Hill continued to believe that muscle was wholly dependent on carbohydrate as an energy source during exercise. This idea was further established by a series of studies of exercising humans performed in the early 1920s by Hill along with Lupton and C.N. Hugh Long (1901-1970) (100-102). In these experiments, Hill and colleagues related changes in blood lactate concentration to changes in $\dot{V}O_2$ during and especially after exercise at various intensities and while breathing various percentages of O_2. In particular, Hill was interested in reconciling the results of these human studies with the results of his and Meyerhof's previous experiments with isolated frog muscle, for which they shared a Nobel Prize in physiology or medicine in 1922. This was achieved via Hill's now well-known O_2 debt hypothesis, which proposed that the initial rapid decline in $\dot{V}O_2$ after exercise represented the oxidation of lactate in the muscles where it was formed whereas the later slower decline in $\dot{V}O_2$ represented the oxidation of lactate after it had escaped from such muscles via diffusion. However, on the basis of the recovery coefficient (i.e., the ratio of CO_2 retained —assumed to equal the amount of lactate oxidized—to O_2 consumed after exercise), it was held that much of the lactate formed during exercise was in fact not oxidized. This dovetailed the results of these human studies with Hill's and Meyerhof's prior calculations that only somewhere between one sixth to one fifth (Meyerhof) or one fourth to one third (Hill) of the lactate produced was oxidized after exercise and that the remainder was resynthesized into its precursor. Hill and Lupton (103) therefore concluded the following:

> The muscle is to be regarded as an accumulator of energy, energy available for rapid nonoxidative discharge, stored during previous oxidations. The transformation of glycogen into lactic acid, the action of the lactic acid on the muscle proteins, and the neutralization of the lactic acid by the alkaline buffers of the muscle, are the vehicle by which this stored energy is made manifest; during recovery the process is reversed at the expense of a portion of lactic acid oxidized. The accumulator has been recharged at the expense of oxidations required to run the dynamo. We must regard the muscle, therefore, as possessing two mechanisms: (a) the anaerobic one of discharge and (b) the oxidative one of recovery. (103, p. 139)

It is not entirely clear why Hill (and many others) continued to believe so strongly that exercising muscle could use only carbohydrate as substrate when the whole-body data of Zuntz, Benedict and Cathcart, and Krogh and Lindhard clearly suggested otherwise. In part, it may have been because studies of rabbit muscle in 1904 by Leathes (122) and frog muscle in 1915 by Winfield (189) failed to demonstrate any reduction in total fatty acid content as a result of electrical stimulation—although Leathes in fact concluded, "It is doubtful whether the utilization of fat in muscular activity can be proved by stimulating the muscles . . . but in other ways it has been established beyond doubt that muscles can and do make use of fat as a source of energy" (122). As well, Hill's misunderstanding may have simply stemmed from his differing conceptualization of the problem at hand. For example, in a lecture delivered to the Mayo Foundation and five universities in the autumn of 1924, he stated:

> It has long been discussed whether the breakdown of carbohydrate, rather than of other substances, is primarily responsible for the provision of energy in muscular contraction. It is known and accepted that work may be done, in the general melting-pot of the body, by the use of any kind of foodstuff. We are now concerned, however, specifically with the primary process of muscular contraction. In the complete chain of processes involved in long-continued exercise, this primary process may be disguised, or even apparently obliterated, by simultaneous transformations that take place between the different food constituents. (97, p. 505)

Thus, Hill's focus was clearly on the molecular events underlying the contractile process itself, which had yet to be elucidated but which he felt were intimately connected to the production of lactate from glycogen. As a result, he (mis)interpreted Krogh and Lindhard's observation of a slightly greater energy cost associated with the oxidation of fat as evidence that "the primary breakdown is of carbohydrate, and that fat is used only in a secondary manner, e.g., to restore the carbohydrate which has disappeared" (97, p. 506), even though the magnitude of the difference that was observed was much less than that predicted by Chauveau or even Zuntz.

The Hill-Meyerhof lactic acid theory of muscle contraction was finally displaced in the late 1920s by a series of breakthroughs that Hill himself described in 1932 as "the revolution in muscle physiology" (98). First among these was the almost simultaneous discovery in 1927 of phosphagen by Philip Eggleton (1903-1954) and Grace Palmer Eggleton (1901-1970) (64) and its identification as creatine phosphate (CrP) by Cyrus H. Fiske and Yellapragada SubbaRow (1895-1948) (68). Both sets of authors found that the compound decreased during exercise and increased during recovery. This, along with its high heat of hydrolysis as soon measured by Meyerhof and Suranyi (138), suggested that it might be an important energy source in muscle. In 1929, Karl Lohman (1898-1978) (124) and Fiske and SubbaRow (69) discovered adenosine triphosphate (ATP), which Lohman correctly hypothesized was the direct source of energy for muscle contraction, with CrP resynthesizing ATP via the famous Lohman reaction: CrP + adenosine diphosphate (ADP) $\leftrightarrow$ Cr + ATP) (125). Finally, in the early 1930s, Einar Lundsgaard (1899-1968) demonstrated that the muscles of frogs poisoned with iodoacetic acid were able to contract despite being unable to produce lactate (126-129), thus driving the final stake into the heart of the Hill-Meyerhof theory of muscle contraction. In 1887, Pohl had reported that the pH of frog muscles treated with bromoacetic acid did not decline during the resultant contracture (150), which in 1924 Schwartz and Oschmann had demonstrated was attributable to the absence of lactate production (162). However, due to the comprehensive nature and timing of Lundsgaard's experiments as well as the fact that they involved electrically induced contractions rather than chemically induced contractures, he is generally credited with bringing the end to the lactic acid era.

These findings, especially Lundsgaard's observations, finally laid to rest the belief that the lactate molecule was directly involved in the contractile process. Other aspects of the O_2 debt hypothesis, however, remained unrefuted. Thus, in 1933, Rodolfo Margaria (1901-1983), Harold T. Edwards (1897-1937), and David Bruce Dill (1891-1986) proposed that the more rapid, or alactacid, part of the biphasic decline in $\dot{V}O_2$ after exercise represented the oxidative resynthesis of ATP and CrP using energy derived from the combustion of "ordinary fuels" whereas the slower, or lactacid, part represented the O_2 cost of oxidizing approximately one tenth of the lactate formed to provide the energy required to resynthesize the remainder into glycogen (131). Margaria and colleagues assumed a value of one tenth rather than one fifth, as suggested by Hill and Meyerhof, because the smaller value was more consistent with their own data and fit with their inherent belief that resynthesis of glycogen from lactate was a highly energetically efficient process. This hypothesis was based on the newly available data described in the pre-

vious paragraph as well as the observation of a close linear relationship between the postexercise lactate concentration and the magnitude of the O_2 debt (but only once the latter exceeded approximately 3 L). Although Margaria and colleagues somewhat pointedly declined to speculate on the nature of the "ordinary fuels" oxidized to repay the alactacid portion of the O_2 debt, they concluded that "the lactacid mechanism has to be considered more like a mechanism of emergency" (131, p. 698) that is called on "only when there may be reasons to believe that the work is carried on in anaerobic conditions" (131, p. 714). The paper by Margaria and colleagues did much to perpetuate the mistaken belief that muscle relied extremely heavily, if not exclusively, on carbohydrate (glycogen) as an energy source during exercise and reinforced the misconception, based in part on studies of the Pasteur effect in yeast, that skeletal muscle produced lactate only when it was hypoxic.

Studies of the role of blood glucose availability in metabolism and fatigue also contributed during this time to the emphasis on carbohydrate as a source of fuel for exercising muscle. For example, in 1924 Levine and colleagues observed that hypoglycemia often developed during long-distance (i.e., marathon) running (123). The following year the same group reported that this hypoglycemia could be prevented, and performance seemingly improved, by ingesting additional carbohydrate before and during exercise (83). Similarly, in 1932 Dill and colleagues reported that when a dog was provided with only water to drink during prolonged treadmill exercise, circulating glucose levels decreased markedly and the animal was unable to continue after 3 to 6.5 h of running (57). However, when fed 20 g of glucose every hour throughout exercise or 40 g at the point of fatigue, the dog could continue running for at least 13 h. Dill and colleagues therefore concluded that fatigue during prolonged exercise was due to lack of bloodborne glucose as a muscular fuel.

Finally, in 1939, E. Hohwü-Christensen (1904-1996) and O. Hansen published a series of papers [which in fact was a supplement to a prior publication by Christensen, Krogh, and Lindhard (35)] in which they readdressed, in part, the issue of how variations in carbohydrate availability induced by different diets influence the mixture of fuels oxidized during exercise (30-34). As previously found by Zuntz (191), Benedict and Cathcart (11), Krogh and Lindhard (118), and others (17, 63), Christensen and Hansen observed that consuming a high-carbohydrate diet for 3 to 7 d beforehand enhanced the rate of carbohydrate oxidation and postponed the onset of fatigue during moderate-intensity exercise. On the other hand, consuming a low-carbohydrate diet for the same period of time markedly reduced the rate of carbohydrate oxidation during exercise and impaired exercise performance; fatigue was accompanied by ketosis and hypoglycemia that were severe enough to result in symptoms of neuroglucopenia. To differentiate between these possible causes of premature fatigue, additional experiments were performed in which two subjects were fed 200 g of glucose at the point of almost complete exhaustion. In response, blood glucose levels quickly increased, the neuroglucopenic symptoms disappeared, and the men were able to exercise for an additional hour. The rate of carbohydrate oxidation, on the other hand, did not change much either before the initial onset of fatigue or after glucose ingestion. In another experiment, one of the men ingested 200 g of glucose 3 h before exercise and then fatigued prematurely due to hypoglycemia and symptoms of neuroglucopenia even though the rate of carbohydrate oxidation was quite high. Although these findings led Christensen and Hansen to conclude, as did Bøje before them (18), that hypoglycemia must cause fatigue by way of its effects on the central nervous system and not by affecting muscle metabolism, the results of these experiments nonetheless dramatically demonstrated the effect of prolonged muscular exercise on bodily carbohydrate stores and especially glucose homeostasis.

By the onset of World War II the following picture had emerged. On the basis of experiments in which RER was measured, it was understood that although the contribution of carbohydrate oxidation to overall energy production increased curvilinearly with increasing exercise intensity, fat oxidation still played a significant role, at least at low to moderate exercise intensities. However, it was generally believed that skeletal muscle itself was heavily, if not exclusively, dependent on carbohydrate as an energy source during exercise and that this need was met by circulating glucose during prolonged aerobic exercise that required much less than 100% of maximal O_2 uptake ($\dot{V}O_2max$) and by glycogen during "violent" exercise that required a greater fraction of $\dot{V}O_2max$. Fatty acids could not be oxidized directly by muscle but could be utilized only after being somehow converted into carbohydrate in other tissues (e.g., the liver). For example, in 1941 Arthur H. Steinhaus (1897-1970) wrote in *Annual Review of Physiology* that "if fats are used as fuel in muscles, it must be by some indirect route" (170, p. 702). Similarly, based on his own research (77) as well as a survey of the literature, in 1942 Gemmill wrote in *Physiological Reviews* that "the results of experiments on fat utilization during muscular work have demonstrated that this substance is used indirectly. There is no experimental evidence at the present time for the direct utilization of fat by mammalian muscle" (78, p. 49). Even Lundsgaard himself adhered to this view, stating in his lecture to the Harvey

Society of New York in 1937 that "it is probable that the high-molecular fatty acids are not attacked, or not readily attacked oxidatively in the muscles" (130, p. 180). Instead, Lundsgaard believed that fatty acids were first converted to ketone bodies in the liver before being metabolized by exercising muscle (discussed later).

Lundsgaard's belief was largely based on experiments performed by his colleague in Copenhagen, Blixenkrone-Møller, in which the clearance of β-hydroxybutyrate by perfused cat hindquarters was measured (16). These studies demonstrated that even resting muscle had a large capacity to metabolize ketone bodies, which increased several-fold during electrical stimulation. Experiments by Barnes and Drury at around the same time also revealed a significant extraction of ketone bodies by dog, rat, and human muscle (10), whereas follow-up studies a few years later by Drury and colleagues in ketotic rats and humans (61) and by Neufeld and Ross in ketotic guinea pigs and humans (143) showed that exercise resulted in a temporary decline in ketone levels in the blood, which was followed by a subsequent increase. This pattern was assumed to reflect an exercise-induced increase in ketone utilization by muscle and ketone production by the liver, the latter becoming evident only during recovery. As a result of these and other studies, Lundsgaard's view of substrate metabolism during exercise became the prevailing wisdom for the next 15 to 20 yr—that is, until the recognition of the importance of nonesterified (free) fatty acids (FFAs) as a source of fuel for muscle (discussed later).

Substrate Utilization During Exercise: Advances From World War II to the 1960s

World War II and its aftermath temporarily slowed the pace of research in exercise physiology. Many budding or already notable scientists took leave of absence from their positions to contribute to the war effort, were forced to relocate, or lost their lives in the conflict. As well, the physical demands of military activity led to a strong shift by exercise physiologists away from studies of substrate metabolism and toward studies of fitness testing, cardiovascular function, heat and altitude acclimatization, ergogenic aids, and so on, as emphasized by C. Taylor and Peter V. Karpovich in their review articles on exercise in *Annual Review of Physiology* in 1945 (174) and 1947 (114), respectively. Notably, Karpovich also lamented that so few physiological studies were conducted in schools of physical education, which he considered the logical place for such research. Although he expressed hope that this situation would soon be remedied such that physical education programs would have a sound scientific basis, it appears to this author that Karpovich's hope has to this day been only partially fulfilled.

On the other hand, research in the more basic fields of biochemistry and muscle physiology forged ahead during the immediate postwar years. It was during this time period, for example, that the details of major metabolic pathways such as the tricarboxylic acid cycle were fully elucidated, aided in part by the availability, in the new nuclear era, of ^{14}C-labeled compounds as described by Sir Hans Krebs (1900-1981) in his 1953 Nobel lecture (117). This increased understanding of basic biochemistry paved the way for an explosion in knowledge regarding metabolism during exercise in the late 1960s and beyond (discussed later). As well, the enhanced availability of radioactively labeled compounds and advances in methods for their analysis and data interpretation saw the use of such materials steadily spread from studies of cell extracts and isolated cells to studies of whole organisms, including exercising humans. This pattern essentially repeated itself in the 1990s with the increased availability of stable isotopically labeled compounds and improved methods for their analysis.

It was also during the decade or so after World War II that biochemists definitively answered the second of two questions posed at the outset of this chapter: What is the immediate source of energy fueling muscle contraction? Since the work of Lohman in the 1920s and 1930s, it had become generally accepted that ATP served this role. Due to the rapid rephosphorylation of ADP by CrP, however, it had not been possible to irrefutably demonstrate a decrease in ATP in response to a single twitch or even in response to repeated muscle contractions except at the point of extreme fatigue. This led Hill to publish his famous paper titled "A Challenge to Biochemists" in 1950 (99) in which he pointedly emphasized this fact and suggested that yet another revolution might still occur and that some other substrate might displace ATP in our understanding in precisely the same way that ATP had displaced phosphagens (CrP) and phosphagens had displaced lactate previously.

To address this issue, Hill proposed that biochemists attempt to measure changes in ATP in response to a tetanus of tortoise muscle in which the very slow speed of contraction (i.e., one fifteenth that of frog muscle) and hence ATP turnover could be further reduced by lowering its temperature to 0 °C to 5 °C. Utilizing precisely this approach, however, neither Münoh-Petersen in 1953 (142) nor Mommaerts and colleagues in 1955 (139) were able to provide absolute proof that ATP was directly utilized. On the other hand, that same year

Table 17.1 Changes in ATP, ADP, and AMP Concentrations in Response to a Single Contraction of Frog Muscle Treated with 1-Fluoro-2,4-dinitrobenzene to Inhibit Creatine Kinase

Condition	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)
Mean value at rest	1.25	0.64	0.10
Mean value after contraction	0.81	0.90	0.24
Change ± SEE*	−0.44 ± 0.05	+0.26 ± 0.02	+0.14 ± 0.03

*n = 9 experiments.
ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate.
Adapted from Cain and Davies 1962.

Lange reported decreases in ATP and CrP and increases in ADP during potassium chloride- and acetylcholine-induced contractures of frog rectus abdominis muscle that had been treated with iodoacetic acid and mustard gas (119). These results, however, were questioned by Davies and colleagues because the contractions took several seconds and significant amounts of adenosine monophosphate (AMP) were found, suggesting that damage to the muscle occurred while it was being removed from its support and processed in a high-speed blender (56). Thus, in 1962 Cain and Davies used 1-fluoro-2,4-dinitrobenzene to potently and irreversibly inhibit creatine kinase in frog muscle, thus enabling them to demonstrate small but significant reductions in ATP and increases in ADP as a result of a single contraction (table 17.1) (22). This study therefore conclusively established, as had been believed for quite some time, that “ATP is the primary energy source and CrP is the secondary energy source for contraction of working muscle” (22, p. 366).

The late 1950s and early 1960s also saw renewed interest in the overall pattern of substrate metabolism during exercise, driven in part by the realization that the FFAs long known to be present in plasma were potentially important energy sources for many tissues rather than artifacts arising from in vitro lipolysis of plasma triglycerides (TG) during sample processing. For example, using measurements of the arteriocoronary sinus difference in O_2 content and FFA concentration in cardiac patients, Gordon and Cherkes in 1956 estimated that up to 70% of the O_2 uptake of the heart was used to oxidize FFAs (84). Using samples provided by Andres and colleagues (3), Gordon and colleagues also measured the arteriovenous difference in FFAs across the forearm of normal volunteers (85). Their analyses revealed a significant extraction of FFAs in 15 out of 22 sample pairs. These observations, coupled with the findings of Andres and colleagues (3) that the uptake of glucose by the forearm could account for only 7% of simultaneously measured O_2 uptake and studies of isolated rat skeletal muscle (e.g., that by Fritz and colleagues in 1958) in which high rates of $^{14}CO_2$ production from ^{14}C-labeled FFAs were observed under a wide variety of conditions, including electrical stimulation (76), finally cemented the importance of FFAs as an energy source for muscle, at least in the basal state.

It was not until 1960, however, that data first emerged that provided clear-cut evidence of increased utilization of FFAs during exercise in humans. In that year, Friedberg and colleagues reported the results of experiments in which a bolus of [1-^{14}C]palmitate was administered to healthy men performing 15 min of vigorous cycling exercise (74). The exercise led to a rapid reduction in plasma FFA concentration, which promptly rebounded during recovery. However, this was not due to a reduction in release of FFAs into the circulation but rather to an increased rate of utilization, as indicated by a near-doubling in the rate of [1-^{14}C]palmitate clearance. Taking into consideration the reduction in plasma FFA pool size, it was estimated that the exercise increased the flux of FFAs through plasma by 25% to 45%. Friedberg and colleagues obtained comparable results during lower-intensity exercise that could be maintained for up to 45 min (75) and used the forearm arteriovenous balance model to demonstrate that contractions increased the rate of conversion of [1-^{14}C]palmitate to $^{14}CO_2$ in human muscle (73). The findings of Friedberg and colleagues were soon confirmed using constant infusion of [1-^{14}C]palmitate in studies reported by Carlson and Pernow in Stockholm (25) and Havel and colleagues in San Francisco (88). Havel then spent 1962 and 1963 on sabbatical in Stockholm, working with Carlson and comparing the use of various tracers (i.e., [1-^{14}C]palmitate, [U-^{14}C]palmitate, [9,10-^{3}H]palmitate, [1-^{14}C]oleate, and [1-^{14}C]linoleate]) to measure FFA turnover during exercise (87). With the exception of [1-^{14}C]linoleate, similar results were obtained regardless of the tracer employed, further establishing the

relevance of the prior (and subsequent) results obtained using palmitate labeled in the first position with ^{14}C (or, later, ^{13}C).

Just as importantly, the results of this and previous studies by Carlson and colleagues and later by others (e.g., 149) using ^{14}C-labeled FFA tracers indicated that although exercise markedly increased the turnover and oxidation of this energy source, FFAs still accounted for only approximately 50% of the fat that was being oxidized, based on data obtained from indirect calorimetry. In an attempt to account for the "missing" fat, Carlson and colleagues first determined the effects of exercise on the rate of clearance of a commercial chylomicron-like TG emulsion from the circulation (24). Observing no change in this rate, Carlson and Fröberg measured the TG and phospholipid contents of the muscles of rats before and after both electrical stimulation and treadmill running (23). Although the phospholipid content of skeletal muscle remained constant in both cases, the TG content was significantly reduced, thus demonstrating the importance of this intramuscular source of lipids during contractile activity. However, due to the difficulty of accurately quantifying small changes in muscle TG levels [as previously recognized by Leathes (122)] or an inadequate increase in metabolic demand [as concluded by Barclay and Stainsby (8)], in 1966 Masoro and colleagues found no change in the TG content of the muscles of monkeys subjected to 5 h of electrical stimulation at 3 Hz (133). The contribution of such lipid stores to overall energy metabolism therefore remained controversial for many years, and only in the past decade or so has any sort of consensus been reached (182).

Thus, with the end of the 1960s approaching the pendulum had seemingly swung so far that FFAs had supplanted not only ketone bodies but even carbohydrate as the primary fuel thought to be used by exercising muscle, at least during aerobic exercise that did not result in a significant increase in lactate production. For example, in 1963 Havel and colleagues (88) concluded that FFAs "are the major circulating metabolites burned by working muscle in the postabsorptive state" (p. 1054), whereas in 1965 Rowell and colleagues (158) wrote, "Although for many years investigators were reluctant to accept the phenomenon of fatty acid oxidation by skeletal muscle, recent evidence has allotted this substrate a major role in skeletal muscle metabolism. Indeed fatty acid oxidation is considered the primary if not the sole energy source in exercising men" (p. 1032). Glycogen was still held to be important during high-intensity exercise but was thought to be mobilized primarily in response to tissue hypoxia, and the resultant production of lactate was thought to limit the contribution of FFAs to energy production by inhibiting lipolysis. On the other hand, other substrates (plasma-borne glucose in particular) were relegated to a relatively minor role. For example, based on experiments in which [U-^{14}C]glucose (which underestimates glucose flux due to tracer recycling) was infused into dogs during prolonged treadmill exercise, Paul and Issekutz (149) concluded that "the rates of turnover and oxidation of plasma glucose play only a minor role in exercise metabolism, and they cannot make up the difference between the energy required to perform the work and the energy supplied by the adipose tissue in form of FFA" (p. 621). Along the same lines, based on studies using the arteriovenous balance approach, Havel and colleagues (89) estimated that "uptake of blood glucose in leg tissues could account for no more than 16% [of total energy expenditure] and a quantity of carbon equivalent to one-third of glucose uptake [is] released as lactate" (p. 95).

Substrate Utilization During Exercise: Advances From the late 1960s to Approximately 1990

The mid- to late 1960s proved to be yet another important watershed in the understanding of exercise metabolism. This was due in part to the reintroduction earlier in the decade of the percutaneous needle biopsy procedure by the Swedish nephrologist Jonas Bergström (1929-2001). Charrière and Duchenne first described the percutaneous needle muscle biopsy technique in 1865 (26). This technique enabled Bergström and his colleagues, primarily Eric Hultman but also others such as Lars Hermansen (1933-1984) and Bengt Saltin (1935–), to directly assess, for the very first time, the effects of exercise on muscle glycogen and high-energy phosphate metabolism in humans (12-14, 108). In one early experiment, for example, they obtained biopsy samples from the vastus lateralis muscles of healthy young men before and after 30 min of moderate-intensity cycle ergometer exercise while also measuring glucose exchange across the leg using the arteriovenous balance method (13). Muscle glycogen content decreased, on average, 29% during exercise, but the arteriovenous glucose difference decreased to 0 or even became negative. These results led Bergström and Hultman (13) to conclude that "muscle glycogen is the main carbohydrate source for muscle activity" whereas "glucose uptake from the blood [is] negligible" (p. 20). In another highly influential study, Bergström and colleagues examined the effects of dietary carbohydrate intake on muscle glycogen levels and exercise perform-

Table 17.2 Pre-Exercise Muscle Glycogen Concentration and Time to Fatigue During Prolonged Exercise in Men Consuming Different Diets

Diet	Muscle glycogen (g/100 g)	Time to fatigue (min)
High protein and fat	0.63 ± 0.10	56.9 ± 1.7
Normal mixed	1.75 ± 0.15	113.6 ± 5.3
High carbohydrate	3.31 ± 0.30	166.5 ± 17.8

Adapted from Bergström et al. 1967.

ance (14). Muscle glycogen stores were found to be markedly increased when strenuous exercise was followed by several days of rest and consumption of a high-carbohydrate diet but were significantly reduced when a low-carbohydrate, high-fat diet was consumed instead. Changes in exercise performance (time to fatigue) closely paralleled these changes in initial muscle glycogen levels on both an individual (i.e., $R = .92$; $P < .001$) and group mean (table 17.2) basis. This frequently cited study provided the basis for the common practice of carbohydrate loading among endurance athletes and did much to establish the primacy of muscle glycogen as an energy source and glycogen depletion as an important factor in fatigue during prolonged exercise.

The window into human muscle metabolism during exercise provided by the biopsy approach also helped catalyze the further growth of exercise physiology research in the United States and helped fuel the careers of investigators such as Philip Gollnick (1934-1991) at Washington State University and David Costill (1932–) at Ball State University. Beginning in 1969, Gollnick and various colleagues used the biopsy method to address questions such as the effects of prolonged exercise on skeletal muscle ultrastructure (80), the pattern of motor unit recruitment during exercise (81), and the effects of endurance training on muscle enzyme activities and fiber type composition (79, 82). Gollnick was also an early leader in the burgeoning field of equine exercise physiology (157). Costill used the biopsy approach to document the metabolic demands of, and adaptations to, endurance exercise, especially the responses to distance running (52, 53). In a key study, Costill and colleagues demonstrated that, in subjects consuming a normal mixed diet, muscle glycogen concentration declined progressively in response to running 16.1 km/d for 3 d and in some subjects did not fully recover after 5 d of rest (53). Combined with the prior observations of Bergström and colleagues (14), the results of Costill and colleagues' study reinforced the importance of muscle glycogen as an energy source and demonstrated the need for endurance athletes to consume adequate dietary carbohydrate during routine training as well as when tapering for an important competition.

Paralleling the expansion of human research that started in the 1960s was a greatly increased interest in the biochemical responses and adaptations of muscle to exercise as studied in animal models, especially rats. This line of research was largely spearheaded by John O. Holloszy (1933–) (figure 17.2), who initially trained as a physician specializing in internal medicine, endocrinology, and metabolism. However, after 2 yr in the U.S. Public Health Service, during which he worked in the Physical Fitness Research Laboratory at the University of Illinois, Holloszy became fascinated by the improvements in performance ability and by the metabolic changes (e.g., reduction in plasma TG) resulting from repeated bouts of exercise. Holloszy therefore returned to Washington University in St. Louis in 1963 to pursue additional training in biochemistry. He joined the faculty in 1965 and for next 10 yr focused on elucidating the biochemical adaptations of skeletal (e.g., 6, 104, 106, 140) and cardiac (147, 177) muscles to chronic exercise. These studies revealed the phenomenal plasticity of skeletal muscle in response to increases in metabolic demand and established the critical importance of muscle mitochondrial content in determining the pattern of substrate utilization during exercise (described in more detail later). Holloszy's research endeavors later expanded to include studies of glucose transport in mammalian (rat) muscle and studies of the metabolic, hormonal, and cardiovascular responses and adaptations to exercise in humans, especially in patient populations such as the elderly or those with type 2 diabetes. For his research, in 2000 Holloszy was awarded the International Olympic Committee's Olympic Prize on Sports Sciences.

The remarkable adaptations observed in rodent skeletal muscle and shortly thereafter in human skeletal muscle (79, 82, 141, 178) in response to increases in physical activity led to renewed attention to the effects of such training on the pattern of substrate utilization

during exercise. Based largely on cross-sectional studies in which RER or blood lactate were measured, it had been generally accepted since early in the century that trained individuals did not rely as heavily on bodily carbohydrate stores during exercise (see 38 for review). However, few if any formal studies of this issue appear to have been performed; most investigations of training focused instead on, for example, the cardiovascular responses to exercise. Indeed, before the 1970s the only longitudinal study of the effects of training on substrate metabolism during exercise appears to be the 1936 report of McNelly (135), who measured the RER of three athletes cycling at a constant intensity before, during, and after a period of training. In 1967, however, Hermansen working with Hultman and Saltin observed that the initial rate of glycogen utilization was 24% lower in trained men than in untrained men during exercise at 77% of $\dot{V}O_2max$ (93). This glycogen-sparing effect of training was confirmed in subsequent cross-sectional and longitudinal studies of both rats (5, 71) and humans (66, 112, 113, 161), thus explaining, at least in part, the already well-known training-induced reductions in RER and blood lactate levels during exercise. Importantly, studies of rats demonstrated that the rate of muscle glycogen utilization during exercise was significantly and inversely correlated with muscle respiratory capacity when the latter varied over a twofold range in groups of rats that were sedentary or had been trained 10, 30, 60, or 120 min/d (73). Conversely, time to fatigue during moderate-intensity treadmill exercise was found to be positively associated with muscle respiratory capacity.

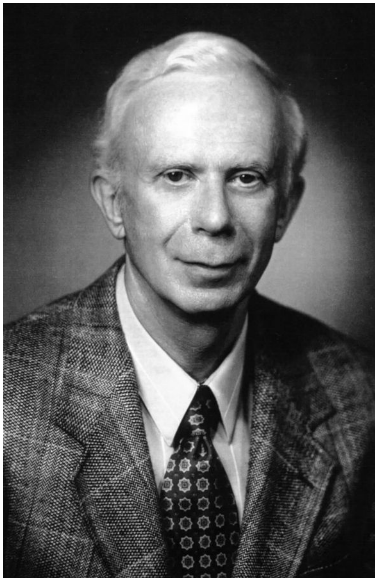

Figure 17.2 John Otto Holloszy (1933–) of Washington University in St. Louis.
Photo courtesy of John O. Holloszy.

These data added further evidence that muscle glycogen is by far the most important energy substrate for exercising muscle and that fatigue is often related to glycogen depletion.

Notably, however, the study by Fitts and colleagues (71) as well as other studies published around the same time (5) also demonstrated that training reduces the rate of utilization of liver glycogen in rats during exercise. In fact, the glycogen-sparing effect of training was even more prominent in the liver than in muscle and was more closely correlated with muscle respiratory capacity (71). Such observations clearly implied that training reduced the dependence of rat muscle on plasma-borne glucose as well as intramuscular glycogen during contractile activity. This hypothesis was confirmed in 1983 by Brooks and Donovan, who used [U-^{14}C]glucose to quantify the turnover and oxidation of glucose in untrained and trained rats during treadmill running (58). On the other hand, previous one-leg training studies conducted in humans failed to show any difference in glucose uptake between the trained and untrained legs during two-leg exercise at 65% to 70% of $\dot{V}O_2max$ (161). Presumably, however, these results reflected the difficulty in accurately quantifying small changes in glucose uptake using the arteriovenous balance method. Thus, in 1990 the present author along with Kohrt, Spina, Bier, and Holloszy used a primed constant infusion of the nonrecycling tracer [U-^{13}C]glucose to measure glucose turnover and oxidation in human subjects before and after 3 mo of endurance-exercise training (43). Consistent with prior studies in rats, this study demonstrated that training reduced the rates of glucose turnover and oxidation by approximately one third (figure 17.3); this decrease in glucose use accounted for roughly one half of the reduction in overall carbohydrate oxidation resulting from training. This preferential sparing of glucose was confirmed in subsequent studies (50, 136), which also demonstrated that this adaptation (a) develops quite rapidly (i.e., within the first 10 d of training) (136) and (b) is evident not only during exercise performed at the same absolute intensity (i.e., the same power output or $\dot{V}O_2$) before and after training but also during exercise performed at the same relative intensity (i.e., the same percentage of $\dot{V}O_2max$) in the untrained and trained states (45). In addition, it has been shown that the training-induced reduction in glucose production during exercise in humans is the result of an attenuated rate of gluconeogenesis as well as a decline in the rate of glycogenolysis (50). This diminished reliance on plasma glucose for energy after training contributes to the training-induced improvement in endurance by protecting against the development of hypoglycemia.

Table 17.3 Effect of Endurance-Exercise Training on Utilization of Intramuscular Triglycerides

Time of biopsy sample	Before training	After training
Before exercise	59.2 ± 7.7	63.3 ± 17.7
After exercise	46.4 ± 8.9	37.2 ± 12.3*
Decrease	12.7 ± 5.5	26.1 ± 9.3†

Values are mean ± standard deviation for n = 9.
Significant difference (after training versus before training): *$P < .05$; † $P < .001$.
Adapted from Hurley et al. 1986.

Due to the emphasis at the time on the importance of plasma-borne FFAs as an energy source for muscle, it was initially assumed that the previously described training-induced reduction in carbohydrate utilization during exercise was compensated for by, and was at least partially due to, an increased uptake and oxidation of FFAs (105, 112). In fact, Issekutz and colleagues reported in 1965 that the rate of FFA turnover in exercising mongrel dogs was inversely related to their blood lactate level and, hence, positively related to their aerobic fitness (111). Similarly, when comparing the results they obtained in untrained men (87) with what they had previously measured in athletes exercising at a similar absolute intensity (88), Havel and colleagues also noted that the trained subjects derived more of their energy from FFAs. Somewhat paradoxically, however, in longitudinal experiments plasma FFA concentrations were observed to increase less during exercise performed at the same absolute intensity after training compared with before training (187, 188). Although in theory this could have been due to a training-induced increase in the rate of FFA clearance from plasma, circulating glycerol concentrations were also observed to be lower after training (187, 188), implying that the reduction in FFA concentration was due to a reduced rate of lipolysis. The authors therefore hypothesized “on the basis of the apparently lower rates of lipid mobilization after training, that the trained individual obtains a greater proportion of his energy requirement during long-term submaximal exercise from intramuscular lipid stores” (188, p. 770). Thus, in 1986, Hurley working in Holloszy’s laboratory measured the TG content of carefully dissected (to remove possible intramuscular adipocytes) muscle biopsy samples obtained from subjects before and after a bout of prolonged exercise performed in the untrained and trained states (109). The results of this study revealed an approximate doubling in the net utilization of this important energy source with training (table 17.3). Other longitudinal and cross-sectional studies using stable isotopic tracers also support the conclusion that training increases the utilization of intramuscular TG during exercise at the same absolute (115, 132, 166) and even the same relative (44) intensity. Under the latter condition, however, the overall rate of lipolysis (as judged from the rate of appearance of glycerol) is greater in the trained state (44, 116), resulting in higher FFA concentrations and hence a greater utilization of this lipid source (44).

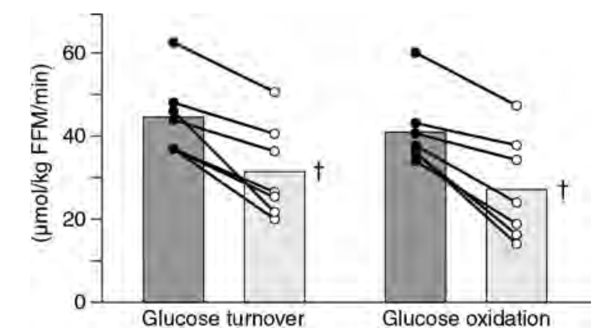

Figure 17.3 Effect of endurance training on plasma glucose turnover and oxidation during prolonged exercise. FFM = fat-free mass. Bars depict group means (dark bars = pretraining; light bars = posttraining); circles (open = pretraining; closed = posttraining) and lines depict individual subjects. †$P < .001$.

Reprinted from A.R. Coggan, 1990, “Endurance training decreases plasma glucose turnover and oxidation during moderate intensity exercise in men,” *Journal of Applied Physiology* 68:990-996. With permission of American Physiological Society.

Along with the effects of training, another major theme of metabolic research during the period between the late 1960s and approximately 1990, especially during the 1980s, was the effect of the ingestion of carbohydrate (and other substrates) during exercise on metabolism and performance. As previously discussed, it had long been accepted that providing glucose to exercising animals or humans could delay fatigue. Based on the studies of Levine and colleagues (123), Dill and colleagues (57), and Christensen and Hansen (33), this delay was generally believed to be due to prevention of hypoglycemia and symptoms of neuroglucopenia. In

1982, however, Felig and colleagues of Yale University reported in *New England Journal of Medicine* that glucose ingestion did not delay fatigue in untrained men exercising at 60% of $\dot{V}O_2max$ (67). This stimulated Coyle (figure 17.4) and colleagues working in Holloszy's laboratory to reinvestigate the effects of carbohydrate feedings during exercise on metabolism and performance (54). Using trained cyclists exercising at a higher intensity (i.e., 75% of $\dot{V}O_2max$) more representative of athletic competition, Coyle and colleagues found that consumption of glucose polymers delayed the onset of fatigue in 7 out of the 10 subjects tested. Because only 2 of the subjects exhibited symptoms of neuroglucopenia, it was hypothesized that postponement of fatigue was due to a muscle glycogen-sparing effect of the feedings. Contrary to this hypothesis, however, in a subsequent study carbohydrate ingestion was found to not alter the rate of muscle glycogen utilization during exercise to fatigue at 70% to 75% of $\dot{V}O_2max$ (55). Instead, it appeared that such feedings delayed fatigue by maintaining plasma glucose availability late in exercise, when muscle glycogen concentrations were very low. Thus, for his dissertation under Coyle's supervision, the present author essentially replicated Christensen and Hansen's classic experimental design by restoring glucose concentrations to normal via either carbohydrate ingestion (40, 41) or glucose infusion (40) late in prolonged exercise or at the point of fatigue.

Carbohydrate ingestion at the point of fatigue often failed to re-establish and maintain euglycemia. On the other hand, both carbohydrate feeding late in exercise and glucose infusion at the point of fatigue did so. This increase in glucose availability was accompanied by an increase in the rate of carbohydrate oxidation and postponement or even reversal of fatigue. Notably, the rate of glucose infusion required to maintain plasma glucose concentrations during continued exercise averaged more than 1.1 g/min and apparently provided more than three quarters of total carbohydrate oxidation during this time period. These data, along with the careful and detailed arteriovenous balance experiments by Felig and Wahren in collaboration with Ahlborg and colleagues at the Karolinska Institute in Stockholm in which the exchange of glucose and other substrates was determined across the legs and splanchnic bed of exercising men (1, 2, 181), helped illustrate the importance of circulating glucose as a source of fuel for muscle, as originally reported by Chauveau and Kaufmann approximately 100 yr before (28). Indeed, it is now well established that plasma-borne glucose normally provides 20% to 50% of total oxidative energy production and 25% to 100% of the total carbohydrate oxidized during prolonged exercise (see 37 for review). Moreover, the studies of Coyle and colleagues also clearly demonstrated that fatigue during prolonged exercise is normally the result of both depletion of muscle glycogen and a decline in plasma glucose availability and is not due to muscle glycogen depletion alone.

Figure 17.4 Edward F. Coyle (seated) of the University of Texas in the United States along with former and current doctoral students Andrew R. Coggan, Scott J. Montain, Marc T. Hamilton, Ricardo Mora-Rodriguez, Ted W. Zederic, and Matthew D. Pahnke (standing, left to right).
Photograph courtesy of Edward F. Coyle.

Substrate Utilization During Exercise: Advances from the Mid-1990s to Present

During the 19th and much of the 20th century, metabolic research in exercise physiology was focused primarily on the acute responses and chronic adaptations of healthy young men. With the approach of the new millennium, however, greater consideration was given to such issues in other subject populations (e.g., women, the elderly, those who are obese or diabetic). As well, ever-increasing attention was paid to unraveling the mechanisms responsible for regulating substrate use in these contexts. These trends were driven in large part by societal issues (i.e., the growing participation of women in sport, the aging of the overall population, and especially the dramatically increasing rates of diseases of inactivity such as obesity and type 2 diabetes) in Western countries and advances in the more basic biological sciences—in the fields of molecular biology and genetics in particular—that led to a more reductionist approach.

As an example of the former trend, in 1990 Tarnopolsky working in MacDougall's laboratory initiated an extensive series of investigations examining the effects of sex on substrate metabolism during and after acute exercise and in response to chronic training (173). Tarnopolsky's studies were not the first in this area but were better controlled than previous research. These

studies established that, compared with men, women tended to rely slightly less on intramuscular glycogen and plasma glucose and slightly more on intramuscular TG and plasma FFAs during endurance exercise. Leucine turnover and oxidation were also found to be lower in women than in men during exercise. Similar results have been obtained by others (159). This difference between men and women in the pattern of substrate utilization during exercise appears to be due in part to a sex hormone-mediated enhancement of the biochemical pathways of lipid mobilization and utilization because short-term administration of 17β-estradiol to men tends to induce similar changes (173). In keeping with this interpretation, in women the rate of plasma glucose utilization and the overall rate of carbohydrate oxidation have been found to be lower during the luteal phase of the menstrual cycle than during the follicular phase (190). Conversely, aging has been found to result in a greater dependence on muscle glycogen and plasma glucose for energy during exercise along with a corresponding reduction in the rate of fat oxidation (163). This appeared to be the result of an age-related reduction in muscle mitochondrial respiratory capacity (39, 48), which impaired the ability of aged muscle to maintain energetic homeostasis during exercise (39). However, both the age-related decrease in muscle enzyme levels (49) and the age-related alterations in substrate metabolism during exercise (164) were at least partially reversible with endurance training, suggesting that they were due to a reduction in habitual physical activity with age and did not reflect a primary effect of aging.

Another area of research that has received considerable attention from exercise physiologists in recent decades has been the effect of obesity or type 2 diabetes on substrate metabolism. Although much of the focus in this area has been on the effects of physical activity on metabolic responses at rest, a number of studies (reviewed in 107) have examined the response of obese or diabetic individuals to exercise. In general, these studies have revealed a marked reduction in the overall capacity of muscle to oxidize fatty acids both at rest and during exercise; this reduction was not reversible upon weight loss (176). At the same time, the uptake of FFAs and their incorporation into intramuscular TG or conversion into downstream metabolites such as ceramide or diacylglycerol appeared to be enhanced in obese persons and type 2 diabetics, likely contributing to the insulin resistance exhibited by such individuals (107).

With regard to mechanisms, in recent years much effort has been expended attempting to elucidate how muscle contractions (or insulin) increase the permeability of muscle to glucose and thereby increase glucose uptake. This has facilitated advances in our understanding of the signaling pathways involved, as recently reviewed by Röckl and colleagues of Harvard University (156). Although much of the interest in this area has originated from its potential importance in insulin-resistant states, the enhanced knowledge regarding the regulation of glucose transport into muscle has greatly increased our understanding of substrate metabolism during exercise. For example, demonstration in 1998 by Hayashi and colleagues (90) of an important role of AMP-activated protein kinase (AMPK) in mediating contraction-induced translocation of glucose transporter 4 (GLUT4) to the sarcolemma provided a potential explanation for the curvilinear relationship between exercise intensity and glucose uptake; both AMPK activity and glucose uptake generally parallel changes in the AMP:ATP ratio in muscle (29). Similarly, attenuation of the exercise-induced increase in AMPK activity with training (134) may explain the somewhat paradoxical finding that even though training increases total muscle GLUT4 content, fewer GLUT4 are translocated to the sarcolemma during exercise in the trained state (155), thus accounting for a slower rate of glucose utilization after training. Training-induced changes in plasma glucose metabolism during exercise are therefore now mechanistically linked to the accompanying alterations in cellular energetics and hence to the increase in mitochondrial respiratory capacity that occurs with training, as originally hypothesized (47). The increase in total GLUT4 that accompanies training therefore appears to be an adaptation aimed more at enhancing the rate of glycogen resynthesis in the postexercise state than at increasing the rate of glucose utilization during exercise itself.

Significant advances have also occurred in the past couple decades in our understanding of the regulation of fatty acid metabolism at the cellular level. For many years it was assumed that FFAs were taken up by muscle and other tissues via simple diffusion across the lipid bilayer of the plasma membrane. This assumption led to the widespread belief that the rate of FFA oxidation during exercise was determined largely, if not entirely, by the plasma concentration of this substrate. When the exercise intensity was low to moderate, adipose tissue lipolysis released FFAs at ever-increasing rates during prolonged exercise, leading to a progressive increase in plasma FFA levels and hence in their rate of oxidation. When the exercise intensity was higher and the duration shorter, inhibition of lipolysis due to an increase in lactate or the accompanying change in pH or entrapment of FFAs in adipose tissue due to redirection of blood flow led to a decline in FFA levels and hence limited their contribution to energy metabolism during exercise. The perspective that exercising muscle is largely a passive consumer of whatever FFAs are presented to it was reinforced in 1963 by the famous glucose–fatty

acid cycle hypothesis of Randle and colleagues (151), which proposed that an increase in FFA availability and hence oxidation inhibited glucose utilization via accumulation of citrate, which inhibited phosphofructokinase and hence caused an elevation of glucose-6-phosphate, which in turn inhibited glucose phosphorylation by hexokinase. This was especially true after Rennie and Holloszy reported that the glucose–fatty acid cycle was operative not only in cardiac and diaphragm muscle as originally reported by Randle and colleagues but also in adequately oxygenated perfused skeletal muscle (154). As a consequence, numerous putative FFA-elevating and hence glycogen-sparing treatments (e.g., heparin injection to activate lipoprotein lipase activity, caffeine ingestion) were studied by various investigators, albeit with decidedly mixed results. The glucose–fatty acid cycle was also often invoked to explain the training-induced reduction in carbohydrate utilization during exercise (105, 112).

Perspectives on the factors regulating lipid utilization by muscle during exercise began to change in the 1990s. This was due in part to the demonstration by Turcotte and colleagues that, as had previously been found in cultured adipocytes, hepatocytes, and cardiac myocytes, uptake of palmitate by perfused skeletal muscle appeared to be saturable when plotted as a function of the unbound instead of the total palmitate concentration (figure 17.5) (177). This clearly implied some sort of transport-mediated process, leading Bonen and colleagues (20) to investigate this issue using a giant sarcolemmal vesicle preparation. As in the perfused hindquarter, vesicular uptake of palmitate was found to follow Michaelis-Menten kinetics (K_m), with a K_m of approximately 6 nM in vesicles prepared from either red or white muscle. On the other hand, the V_{max} of palmitate uptake was approximately twice as high in vesicles from red muscle as in vesicles from white muscle, in keeping with the vesicles from red muscle's approximately twofold greater content of the putative fatty acid transporters fatty acid translocase (FAT/CD36) and plasma membrane fatty acid binding protein ($FABP_{PM}$). Subsequent studies firmly established that the uptake of FFA by skeletal muscle is acutely regulated in a manner quite similar to the uptake of glucose (i.e., via the translocation of transport proteins from intracellular storage pools to the sarcolemma and back again, as reviewed by Bonen and colleagues; 19). Moreover, endurance training has been recently shown to enhance the capacity of skeletal muscle to metabolize FFAs, in part by increasing the expression of FAT/CD36 and $FABP_{PM}$ (172). Thus, rather than being an entirely passive process, FFA uptake by muscle is now recognized as being under both short-term and long-term control. At the same time, other studies have shown that although muscle citrate levels are higher during exercise in the trained state (46, 112), glucose-6-phosphate concentrations are actually lower (46, 51), demonstrating that "the training-induced reduction in carbohydrate utilization during prolonged submaximal exercise occurs by modification of the glycogenolytic/glycolytic flux before the phosphofructokinase step and is not due to inhibition of phosphofructokinase by citrate or by other metabolites" (46, p. E220). Thus, although the glucose–fatty acid cycle appears to operate in exercising skeletal muscle under at least some conditions (154), this mechanism does not explain the lesser dependence on muscle glycogen and plasma glucose during exercise in the trained state.

Finally, data obtained in the past two decades have revealed new pathways by which carbohydrate and lipid metabolism interact in various tissues, including exercising skeletal muscle. In particular, significant attention has been focused on the role of malonyl-CoA, the product of acetyl-CoA carboxylase and a potent inhibitor of carnitine palmityltransferase I (CPT-I). Based on studies demonstrating that CPT-I in skeletal muscle is especially sensitive to inhibition by malonyl-CoA, in 1989 Winder and colleagues assessed changes in muscle malonyl-CoA levels in response to acute treadmill exercise in rats (185). Malonyl-CoA levels were found to decrease by nearly two thirds, thus presumably disinhibiting CPT-I and contributing to the exercise-induced increase in fat oxidation. Subsequent studies from Winder's group revealed that the decline in malonyl-CoA occurs in a time- (186) and intensity- (152) dependent manner during exercise but was attenuated by the infusion of glucose (65). Observations such as these led to the proposal that a reverse glucose–fatty acid cycle is operative during exercise (i.e., that increases or decreases in the rate of carbohydrate oxidation decrease or increase the rate of fat oxidation via changes in malonyl-CoA) (152, 165, 184). This hypothesis is seemingly supported by the results of studies in which [1-^{14}C]octanoate and [1-^{13}C]palmitate were infused simultaneously into exercising humans to quantify the relative oxidation of medium- and long-chain fatty acids, respectively (166, 167). In these studies, the percentage of the long-chain fatty acid tracer that was oxidized decreased with increasing exercise intensity in untrained individuals (167) but was higher in trained subjects than in untrained subjects (166). On the other hand, the percentage oxidation of the medium-chain fatty acid tracer did not vary significantly across conditions, which was interpreted to mean that the differences in long-chain fatty acid oxidation were due to inhibition of CPT-I, presumably by malonyl-CoA. In contrast to the [1-^{13}C]palmitate, however, it now seems likely that the vast majority of the [1-^{14}C]octanoate was

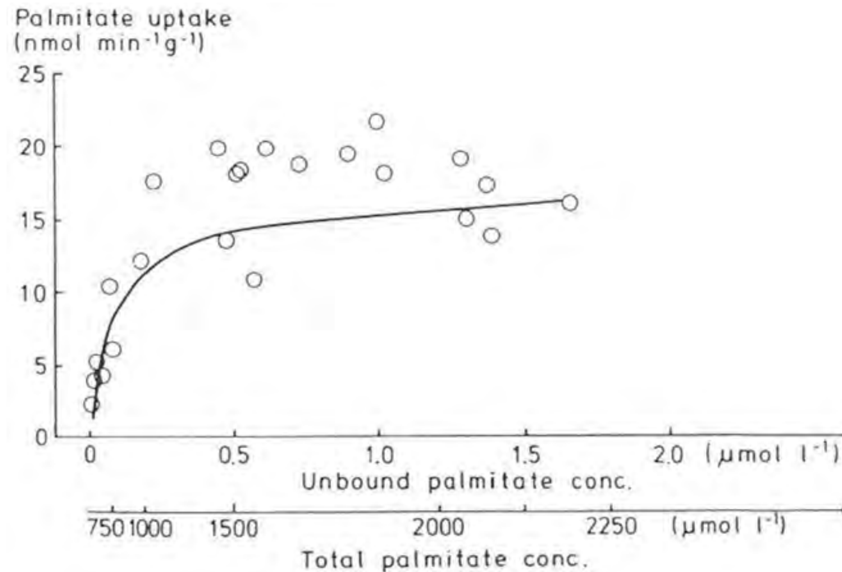

Figure 17.5 Relationship of palmitate uptake to unbound palmitate concentration in perfused rat hindquarters.

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oxidized by tissues other than skeletal muscle (presumably the liver), making the results of these whole-body studies difficult to interpret (42). Furthermore, other studies have demonstrated that malonyl-CoA levels in human muscle change only transiently, if at all, during exercise (144, 145). Finally, the sensitivity of muscle CPT-I to inhibition by malonyl-CoA is even higher in trained persons than in untrained persons (169). Hence, in humans the reciprocal relationship that exists between carbohydrate and fat utilization during exercise both before and after training must be due to some mechanism other than malonyl-CoA-mediated changes in CPT-I activity. As recently reviewed by Sahlin (160), a number of alternative hypotheses have been proposed: inhibition of CPT-I by lactate or H^+; reduced flux through CPT-I due to a decline in free carnitine levels, stemming from overproduction of acetyl-CoA via pyruvate dehydrogenase and hence an increase in acetyl carnitine; a reduction in beta oxidation due to the resultant increase in the acetyl-CoA:free CoA ratio; and inhibition of beta oxidation by NADH produced via metabolism of pyruvate-derived acetyl-CoA in the tricarboxylic acid cycle. Further research is required to fully elucidate the precise mechanisms involved. Regardless, it now seems clear that the rate of carbohydrate utilization during exercise largely dictates the rate of fat utilization, not the reverse as originally believed. In turn, this implies that the increase in fat oxidation with training is primarily the result of the reduction in carbohydrate use, not vice versa as initially hypothesized (105, 112).

Summary

Table 17.4 lists some of the most significant studies of substrate metabolism during acute and chronic exercise that have been published during the past century. These studies, along with the other investigations mentioned in this chapter as well as experiments far too numerous to discuss, have yielded a highly detailed understanding of the pattern of substrate utilization during exercise. Consequently, the first question posed at the outset of this chapter (i.e., what substrates are in fact oxidized during exercise?) seems to have been largely answered. In contrast to what had been widely believed early in the century, it is now well established that no single substrate provides the energy to support muscle contraction. Rather, exercising muscle has been revealed to be a metabolic omnivore, utilizing both carbohydrate and fat derived from intramuscular stores and delivered via the plasma. The precise mix depends on a wide variety of factors, including the intensity, duration, and mode of exercise; the environmental conditions; and the habitual diet, nutritional state (i.e., fasted vs. fed), training status, sex, age, and health status of the individual in question. Carbohydrate in general (and muscle, especially in untrained individuals or during high-intensity exercise, with fatigue muscle glycogen in particular), however, still seems to be the preferred substrate of exercising during prolonged intense exercise that often corresponds to depletion of bodily carbohydrate stores.

Table 17.4 Historically Significant Studies of the Effects of Acute and Chronic Exercise on Substrate Metabolism (1910-Present)

Year	Authors and reference	Findings and significance
1913	Benedict and Cathcart (11)	Demonstrated that respiratory exchange ratio varied markedly with pre-exercise diet but that efficiency did not, thus providing evidence against Chauveau's hypothesis that fat must first be converted into carbohydrate before being oxidized by muscle.
1920	Krogh and Lindhard (118)	Confirmed the results of Benedict and Cathcart.
1923	Hill and Lupton (103)	Proposed the O_2 debt hypothesis linking changes in postexercise O_2 consumption to lactate oxidation.
1924	Levine at al. (123)	Observed that hypoglycemia often developed during prolonged running.
1927	Eggleton and Eggleton (64); Fiske and SubbaRow (68)	Eggleton and Eggleton discovered phosphagen, which Fiske and SubbaRow almost simultaneously identified as creatine phosphate.
1929	Lohman (124); Fiske and SubbaRow (69)	Lohman and Fiske and SubbaRow identified adenosine triphosphate, which Lohman correctly hypothesized was the direct source of energy for muscle contraction.
1930-1932	Lundsgaard (126-129)	Demonstrated that frog muscles poisoned with iodoacetic acid were able to contract despite being unable to produce lactate, thus finally refuting the Hill-Meyerhof theory of muscle contraction.
1932	Dill et al. (57)	Demonstrated in dogs that prolonged exercise led to hypoglycemia and fatigue, which could be prevented or reversed by feeding glucose throughout exercise or at the point of exhaustion, thus cementing the link between carbohydrate availability and exercise performance.
1933	Margaria et al. (131)	Modified Hill and Lupton's original O_2 debt hypothesis to include an alactacid component reflecting creatine phosphate resynthesis as well as a lactacid component due to lactate oxidation.
1936	McNelly (135)	Was the first to demonstrate, using a longitudinal study design, that endurance training reduces the rate of carbohydrate oxidation and increases the rate of fat oxidation during exercise at the same absolute intensity.
1938	Blixenkrone-Møller (16)	Demonstrated that resting muscle had a large capacity to metabolize ketone bodies, which increased several-fold during contractions. Combined with prior observations, this contributes to the widespread belief that muscle utilizes fatty acids only after they are partially oxidized in the liver.
1939	Christensen and Hansen (30-34)	Published a series of papers confirming and extending the earlier research of Benedict and Cathcart, Krogh and Lindhard, and Dill and colleagues.
1950	Hill (99)	Issued his famous challenge to biochemists that questioned whether adenosine triphosphate is in fact the direct source of energy during muscle contractions.
1957	Gordon et al. (85)	Demonstrated significant extraction of FFAs across the forearm of resting humans.

1958	Fritz et al. (76)	Using [1-^{14}C]palmitate, demonstrated increased oxidation of FFAs by isolated rat muscle in response to electrical stimulation.
1960	Friedberg et al. (74)	Using [1-^{14}C]palmitate, demonstrated that exercise increases the flux of FFAs through plasma in humans.
1961-1964	Carlson and Pernow (25); Havel et al. (88); Havel et al. (87)	Performed extensive studies using constant infusion of [1-^{14}C]palmitate and other ^{14}C-labeled fatty acid tracers during exercise, thus firmly establishing the importance of FFAs as an energy source.
1962	Cain and Davies (22)	Used 1-fluoro-2,4-dinitrobenzene to inhibit creatine kinase in frog muscle, making it possible to demonstrate changes in adenosine triphosphate during a single contraction and thus answering Hill's challenge.
1963	Randle et al. (151)	Proposed the glucose–fatty acid cycle by which oxidation of FFAs inhibits glycolysis and, hence, glucose uptake and oxidation via citrate-mediated inhibition of phosphofructokinase activity. This concept strongly influenced beliefs regarding the regulation of substrate metabolism during exercise for several decades.
1966-1971	Bergström and Hultman (13); Bergström et al. (14); Holloszy (106); Molé and Holloszy (140)	Bergström and colleagues used the muscle biopsy technique to quantify changes in muscle glycogen concentration during exercise in humans and demonstrated a very close relationship between initial glycogen levels and time to fatigue during prolonged exercise performed after low, moderate, or high carbohydrate intake. These findings emphasized the critical importance of muscle glycogen even during submaximal exercise and provided the scientific basis for the practice of carbohydrate loading among athletes. Holloszy and colleagues performed detailed studies of the effects of endurance training of rats on muscle mitochondrial respiratory capacity and enzyme activities, thus revealing the remarkable plasticity of muscle in response to alterations in chronic use.
1974-1975	Karlsson et al. (113); Baldwin et al. (5); Fitts et al. (71)	Using a longitudinal study design, Karlsson and colleagues demonstrated the muscle glycogen-sparing effect of endurance training in humans, thus providing a mechanistic explanation for the reduction in respiratory exchange ratio first observed by McNelly. Working in Holloszy's laboratory, Baldwin and colleagues demonstrated that training of rats reduces utilization of both muscle glycogen and liver glycogen during exercise. This diminished reliance on such stores is closely correlated with the training-induced increase in muscle respiratory capacity.
1977	Rennie and Holloszy (154)	Confirmed operation of Randle's glucose–fatty acid cycle in skeletal muscle, thus reinforcing the perspective that FFA availability was a primary determinant of the pattern of substrate utilization during exercise.
1986	Hurley et al. (109); Coyle et al. (55)	Hurley and colleagues were the first to demonstrate that the training-induced increase in fat oxidation during exercise is due, at least in part, to an increased reliance on intramuscular triglycerides. Coyle and colleagues reaffirmed the results of prior studies demonstrating that carbohydrate feedings during prolonged exercise can delay fatigue; however, they demonstrated that this was not due to a sparing of muscle glycogen.

(continued)

Table 17.4 *(continued)*

Year	Authors and reference	Findings and significance
1987	Coggan and Coyle (40); Constable et al. (51)	Coggan and Coyle confirmed Christensen and Hansen's classic observations that restoring plasma glucose availability late in exercise can reverse fatigue and showed that this was associated with an increase in carbohydrate oxidation. They also demonstrated using the euglycemic clamp technique that the rate of glucose uptake can exceed 1 g/min during the latter stages of prolonged intense exercise, thus helping reestablish the importance of this energy source. Constable and colleagues demonstrated that the training-induced reduction in carbohydrate utilization during electrical stimulation of rat muscle was accompanied by a reduction in glucose-6-phosphate levels, thus undermining the hypothesis that endurance training affects substrate use via Randle's glucose–fatty acid cycle.
1989-1990	Winder et al. (185, 186)	Reported that muscle malonyl-CoA levels decrease during exercise in rat muscle, thus relieving inhibition of carnitine palmitoyltransferase I and thereby increasing fatty acid oxidation.
1991	Turcotte et al. (177)	Demonstrated that FFA uptake by perfused muscle exhibits saturation kinetics when the rate of uptake is plotted as a function of the free instead of the total (predominantly albumin bound) FFA concentration, implying that FFAs are taken up via a specific transport mechanism instead of via simple diffusion as previously believed.
1993	Coggan et al. (46)	Demonstrated that muscle glucose-6-phosphate concentrations are lower in humans during prolonged exercise after training compared with before training even though muscle citrate concentrations are higher. This confirmed Constable's prior observations in electrically stimulated rat muscle and definitively ruled out the glucose–fatty acid cycle as the mechanism by which training reduces carbohydrate use during exercise.
1996	Odland et al. (144)	Reported that, in contrast to the responses observed in rat muscle, malonyl-CoA does not decrease during exercise in humans, thus indicating that other mechanisms must be responsible for regulating fatty acid oxidation in exercising humans.
1998	Hayashi et al. (90)	Demonstrated the importance of adenosine monophosphate-activated protein kinase in regulating glucose transport during contractions, thus providing a mechanistic link between alterations in cellular energetic and glucose uptake.
1998	Bonen et al. (20)	Used giant sarcolemmal vesicle preparation to demonstrate that palmitate transport into skeletal muscle follows Michaelis-Menten kinetics: The twofold difference in V_{max} between red and white muscle paralleled the twofold difference in fatty acid translocase (FAT/CD36) and plasma membrane fatty acid binding protein ($FABP_{PM}$). This study, along with the previous work of Turcotte and colleagues (177) and subsequent experiments, helped establish that the transport of FFAs into muscle is actively regulated in a manner similar to glucose rather than being due to passive diffusion.

FFA = free fatty acid.

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