Chapter 11

Carbohydrate Metabolism During Exercise

Kelly M. Hammond, Marc J. Fell, Mark A. Hearris and James P. Morton
Research Institute for Sport and Exercise Sciences Liverpool John Moores University, Liverpool, UK

11.1 INTRODUCTION

The study of carbohydrate (CHO) metabolism in relation to sport and exercise is a field of investigation that is now over 100 years old. Indeed, almost a century ago, Krogh and Lindhard (1920) reported the efficiency of CHO as a fuel source during exercise and also demonstrated that fatigue occurs earlier when subjects consume a high-fat diet (as compared with a high CHO diet) in the days preceding an exercise bout undertaken at a fixed workload. Levine et al. (1924) also observed that runners who completed the 1923 Boston marathon exhibited hypoglycemia immediately postexercise, thus, suggesting that low CHO availability may be linked to fatigue. These early studies provided the initial evidence that CHO was an important fuel source for exercise performance.

Nonetheless, much of the foundation of our understanding of CHO metabolism was developed by Scandinavian researchers in the late 1960s with the introduction of the muscle biopsy technique (Bergstrom and Hultman, 1966; Bergstrom et al., 1967; Hermansen et al., 1967). These researchers provided the platform for modern day sports nutrition practice in a series of studies that collectively demonstrated: (1) muscle glycogen is depleted during exercise in an intensity-dependent manner; (2) high CHO diets increase muscle glycogen storage and subsequently improve exercise capacity; and (3) muscle glycogen storage is enhanced following prior glycogen depletion (i.e., the super-compensation effect), the magnitude of which is dependent on high CHO availability. This body of work remains some of the most highly cited papers in the field and is referenced accordingly in contemporary sport nutrition guidelines (Thomas et al., 2016).

The field continued to develop throughout the 1980s and 1990s with the consistent finding that CHO feeding during exercise also improved exercise performance and capacity (Coyle et al., 1986; Bosch et al., 1994; Tsintzas et al., 1995; Jeukendrup et al., 1997; Jeukendrup and Jentjens, 2000). Such studies relied on the use of stable isotope methodology (to quantify exogenous CHO oxidation) as well as magnetic resonance imaging to quantify liver glycogen depletion during exercise (Casey et al., 2000). As such, it is now generally accepted that liver glycogen depletion is also a major contributing cause of fatigue during endurance exercise. It is noteworthy, however, that CHO feeding can also improve performance via nonmetabolic effects through modulating regions of the brain associated with reward and motor control (Carter et al., 2004a,b; Chambers et al., 2009).

In addition to a simple “fuel store,” our understanding of CHO metabolism has advanced considerably with the use of more sophisticated molecular biology techniques. In this regard, it is now accepted that glycogen is more than a store (Philp et al., 2012), acting as a regulator of many key cell-signaling pathways related to promoting the oxidative phenotype, insulin sensitivity, contractile processes, obesity, protein degradation, and autophagic processes (Philp et al., 2012; Bartlett et al., 2015). When taken together, it is remarkable that whole-body storage of only 500 g of substrate can exert such profound effects on multiple tissues, organs, and systems, the result of which has considerable effects to human health and performance.

The aim of this chapter is to therefore present a contemporary review of our understanding of CHO metabolism with specific reference to exercise metabolism and physiology. We begin by presenting an overview of CHO storage followed by outlining regulatory steps in the control of both muscle glycogen metabolism and muscle glucose uptake. We then proceed to discuss how manipulating substrate availability (i.e., CHO availability itself)

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and alterations to specifics of the exercise protocol (e.g., intensity, duration) and training status of the athlete can all affect the magnitude of CHO utilized during exercise. The previous section, therefore, provides the platform to discuss the well-known effects of both endogenous (i.e., liver and muscle glycogen) and exogenous (i.e., CHO feeding during exercise) CHO availability on exercise performance. Finally, we then discuss the role of CHO availability on modulating aspects of training adaptation, a field of research that has grown rapidly in the last decade. Due to space constraints, it is not possible to review all papers in the field, though we have chosen to highlight and integrate those seminal papers that have significantly advanced our understanding of both metabolic regulation and practical application.

11.2 OVERVIEW OF CARBOHYDRATE STORAGE

CHO is predominantly stored as glycogen in both the liver (approximately 100 g) and muscle (approximately 400 g) with 5 g also circulating in the bloodstream as glucose. In skeletal muscle, glycogen is typically expressed as $mmol \cdot kg^{-1}$ of dry muscle weight (d.w.) where concentrations in whole muscle homogenate can vary from 50 to 800 $mmol \cdot kg^{-1}$ depending on training, fatigue, and dietary CHO intake (see Fig. 11.1).

The glycogen granule itself is essentially a tiered structure of glucose units (i.e., polymers) that is formed in a branch-like structure via 1:4 and 1:6 glycosidic bonds. Glycogen granules are formed on the protein glycogenin and can be as large as 42 nm in diameter as well as having potentially 12 tiers. At its maximal size, the granule can consist of as much as 55,000 glucosyl units (Graham et al., 2008). Nonetheless, the majority of glycogen granules in human skeletal muscle are reported to be 25 nm in diameter with approximately 8 tiers (Marchand et al., 2002). Although muscle glycogen has traditionally been quantified through acid hydrolysis in whole muscle homogenate, it is of course apparent that glycogen is expressed and utilized in fiber type-specific patterns as well as being located in specific intracellular locations within muscle cells themselves. Using histochemical techniques, it has typically been reported that resting glycogen content is not apparently different between type I and type II fibers (Essen and Henriksson, 1974; Essen et al., 1975; Stellingwerff et al., 2007). Nonetheless, using biochemical quantification (a more quantitative measure) it has been reported that type II fibers may contain 50–100 $mmol \cdot (kg\ d.w.)^{-1}$ more glycogen than type I fibers (Tsintzas et al., 1995, 1996). Regardless of method of quantification, glycogen depletion during exercise is dependent on fiber type recruitment patterns depending on the specifics of the exercise protocol. For example, during prolonged steady-state type protocols, type I fibers show a preferential depletion whereas during near maximal or supra-maximal type activity, type II fibers become recruited and show considerable glycogen depletion (Gollnick et al., 1974). In activities involving high-intensity intermittent exercise (e.g., a soccer match), considerable

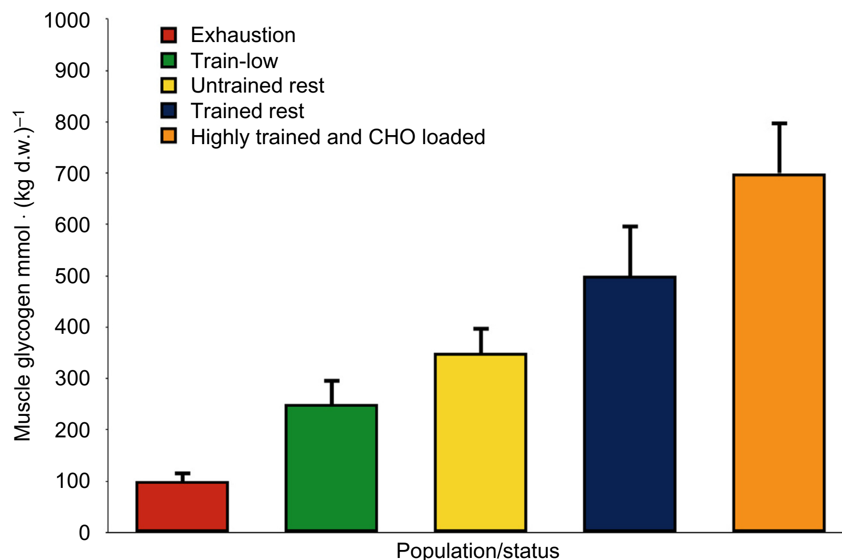

FIGURE 11.1 Variations in muscle glycogen storage according to fatigue status, training status, and dietary CHO intake. *Data are compiled from studies including Bussau, V.A., et al., 2002. Eur. J. Appl. Physiol. 87, 290–295, Bartlett, J.D., et al., 2012. J. Appl. Physiol. 112, 1135–1143, Taylor, C., et al., 2013. Eur. J. Appl. Physiol. 113, 1457–1468, Impey, S.G., et al., 2015. Physiol. Rep. 4, e12803 (taken from Fig. 11.2, Hearris, et al., 2018. Nutrients 10, E298, under the terms of the Creative Commons Attribution 4.0 International License, https://creativecommons.org/licenses/by/4.0/).*

glycogen depletion is observed in both muscle fiber types thus reflecting recruitment patterns to support both moderate and high-intensity running speeds (Krustrup et al., 2006).

The use of transmission electron microscopy (TEM) has also revealed that glycogen is stored in three distinct subcellular pools contained in the myofibrils (intramyofibrillar glycogen, 5%–15% of total glycogen pool), between myofibrils (intermyofibrillar glycogen, 75% of total glycogen pool) and also beneath the sarcolemmal region (sub-sarcolemmal glycogen, 5%–15% of total glycogen pool). In endurance-trained athletes, it appears that both intramyofibrillar and sub-sarcolemmal glycogen stores are greater in type I fibers compared with type II fibers whereas inter-myofibrillar glycogen storage is greater in type II fibers (Nielsen et al., 2011). In relation to acute exercise itself, it is also apparent that intramyofibrillar glycogen stores show a preferential depletion (Marchand et al., 2007) and that failure to restore this specific pool in the immediate hours after exercise is associated with impaired Ca^{2+} release from the sarcoplasmic reticulum (SR) (Nielsen et al., 2011; Ortenblad et al., 2011). Clearly, our understanding of muscle glycogen storage has advanced considerably and there remains a definitive need to further quantify intracellular glycogen utilization in a variety of exercise settings, according to training status, age, and gender.

11.3 REGULATION OF CARBOHYDRATE METABOLISM

An overview of key steps in the regulation of CHO metabolism is provided in Fig. 11.2. There are a number of potential sites of control that can regulate the interaction of CHO and lipid metabolism during endurance exercise. These include availability of intramuscular and extra-muscular substrate (controlled by diet and the action of key hormones such as the catecholamines and insulin), the abundance of transport proteins involved in transporting substrates across both the plasma and mitochondrial membranes and, of course, the activity of the key regulatory enzymes involved in the metabolic pathways. The activity of regulatory enzymes can be modified acutely through covalent modification (i.e., phosphorylation and dephosphorylation largely under hormonal control) and/or allosteric regulation via important signaling molecules that are produced in the muscle as a result of contraction,

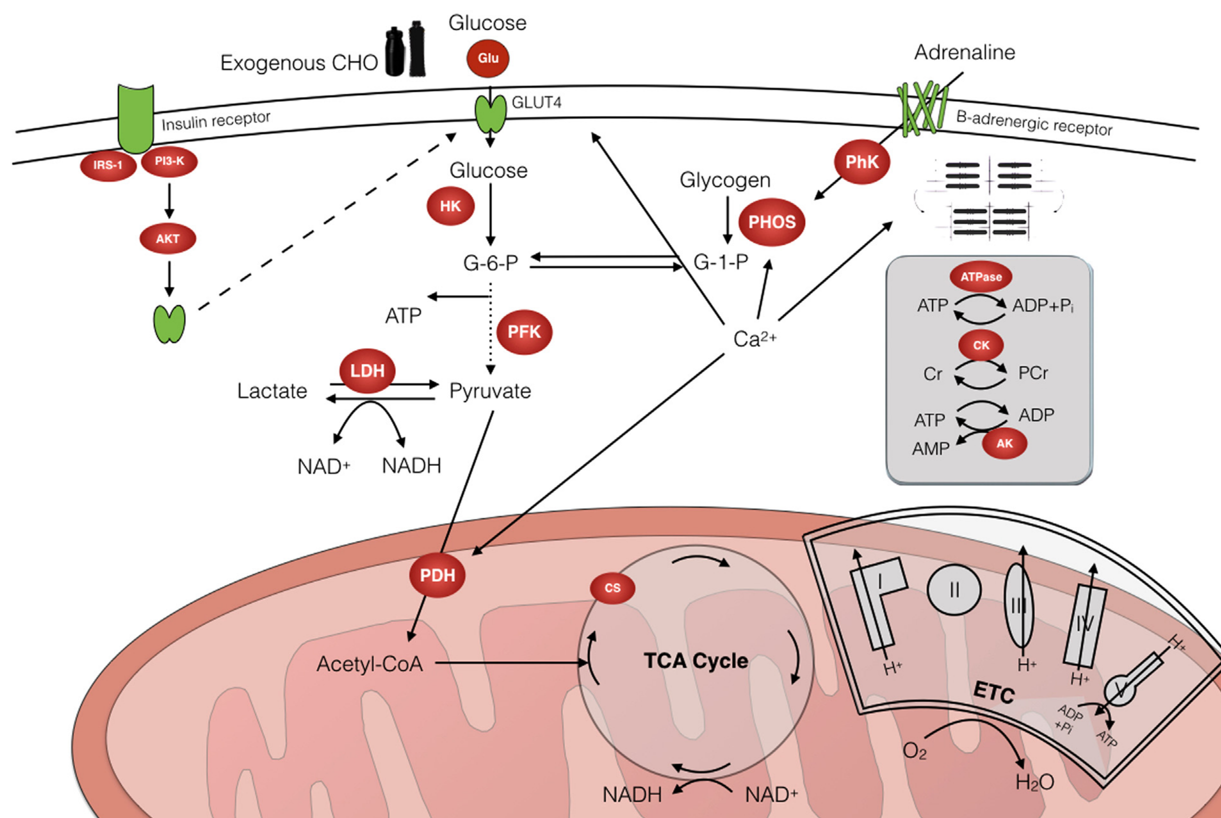

FIGURE 11.2 Overview of CHO metabolism and main control points. Key regulatory enzymes are well recognized as PHOS, HK, PFK, LDH, and PDH. Additionally, the rate of muscle glucose uptake can also determine the flux through glycolysis. *Taken from Fig. 11.2, Hearris, M.A., et al., 2018. Nutrients 10, E298, under the terms of the Creative Commons Attribution 4.0 International License, https://creativecommons.org/licenses/by/4.0/.*

for example, ADP, AMP, IMP, Pi, Ca^{2+}, and H^+. Enzyme activity can also be modified through substrate activation or product inhibition such that increasing the substrate concentration increases catalysis whereas increased product concentration may inhibit the reaction. Finally, enzyme activity can be regulated long term through increasing the muscle cell's content of the actual enzyme protein (i.e., more of the enzyme is actually present) as would occur with endurance training. Clearly, muscle cells possess a highly coordinated and regulatory network of signaling and feedback pathways which function to ensure ATP demand is matched by ATP synthesis. From a physiological perspective, key factors such as exercise intensity, duration, nutritional status, training status, etc. can all regulate substrate utilization during exercise, largely through influencing the potential regulatory control points discussed earlier. This section will outline the regulation of CHO utilization during endurance exercise where we pay particular attention to what is *currently* considered the predominant sites of regulation that is relevant to the specific situation. As a prelude to the text to follow, it is pertinent to highlight that major metabolic control points include glycogen phosphorylase (see Fig. 11.3), muscle glucose uptake (see Fig. 11.4) and pyruvate dehydrogenase (PDH) (see Fig. 11.5).

ADP, adenosine diphosphate; AK, adenylate kinase; Akt, protein kinase B; AMP, adenosine monophosphate; ATP, adenosine triphosphate; Ca^{2+}, calcium; CHO, carbohydrate; CK, creatine kinase; Cr, creatine; CS, citrate synthase; ETC, electron transport chain; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; Glu, glucose; GLUT4, glucose transporter 4; H^+, hydrogen ion; H_2O, water; IRS-1, insulin receptor substrate 1; HK, hexokinase; LDH, lactate dehydrogenase; O_2, oxygen; NAD, nicotinamide adenine dinucleotide; TCA cycle, tricarboxylic acid cycle; Pi, inorganic phosphate; PCr, phosphocreatine; PFK, phosphofructokinase; PhK, phosphorylase kinase; Phos, glycogen phosphorylase; PI3-K, phosphoinositide 3-kinase.

11.3.1 Effects of Exercise Intensity and Duration

As exercise intensity progress from moderate (i.e., 65% $\dot{V}O_{2max}$) to high-intensity (85% $\dot{V}O_{2max}$), muscle glycogenolysis and glucose uptake increases such that CHO metabolism predominates. In contrast, there appears to be reduction in whole-body lipid oxidation due to a reduction in both plasma FFA and intramuscular triglyceride oxidation. Maximal rates of lipid oxidation are considered to occur around 65% $\dot{V}O_{2max}$ though this is dependent on a number of other factors such as training status, gender, and diet (Achten and Jeukendrup, 2004).

The breakdown of muscle glycogen to glucose-1-phosphate is under the control of glycogen phosphorylase and this reaction requires both glycogen and Pi as substrates. Phosphorylase, in turn, exists as a more active *a* form (which is under the control of phosphorylation by phosphorylase kinase) and also as a more inactive *b* form

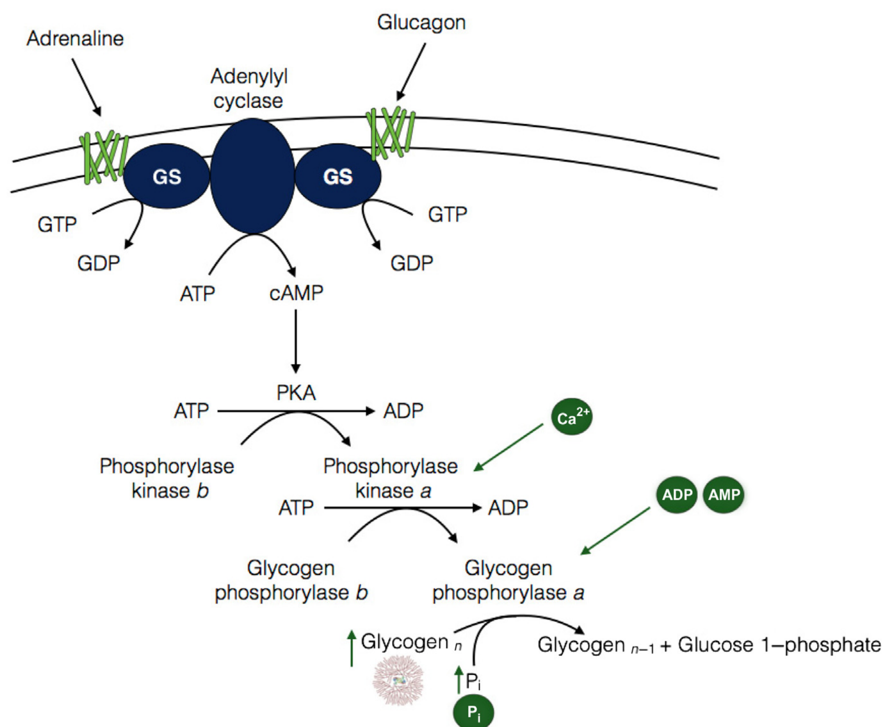

FIGURE 11.3 Regulation of glycogen phosphorylase activity. Positive allosteric effectors are shown in green.
ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; Ca^{2+}, calcium; cAMP, cyclic adenosine monophosphate; GS, G protein; GDP, guanosine diphosphate; GTP, guanosine triphosphate; PKA, protein kinase A; Pi, inorganic phosphate.

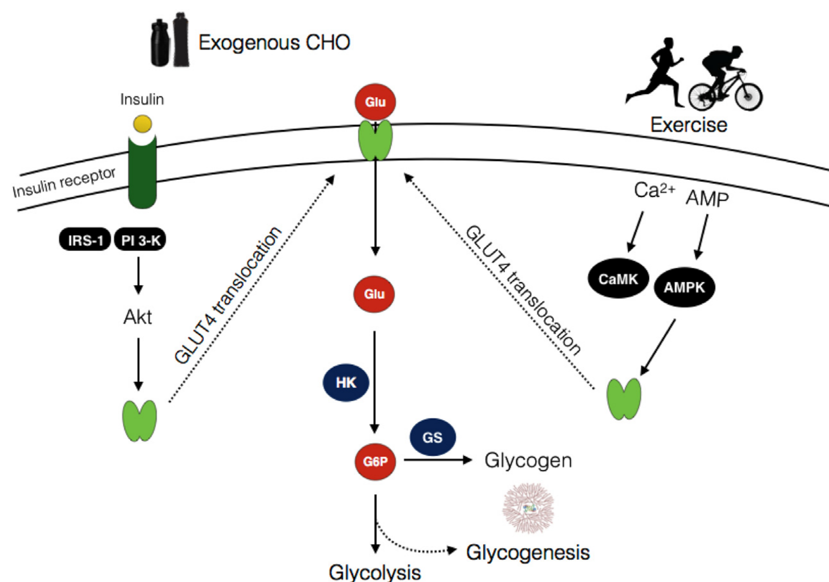

FIGURE 11.4 Regulation of muscle glucose uptake.
AMP, adenosine monophosphate; AMPK, 5′ adenosine monophosphate-activated protein kinase; Akt, protein kinase B Ca^{2+}, calcium; CaMK, calmodulin-dependent protein kinase; G-6-P, glucose-6-phosphate; GS, glycogen synthase; HK, hexokinase; IRS-1, insulin receptor substrate 1; PI 3-K, phosphatidylinositol 3-kinase.

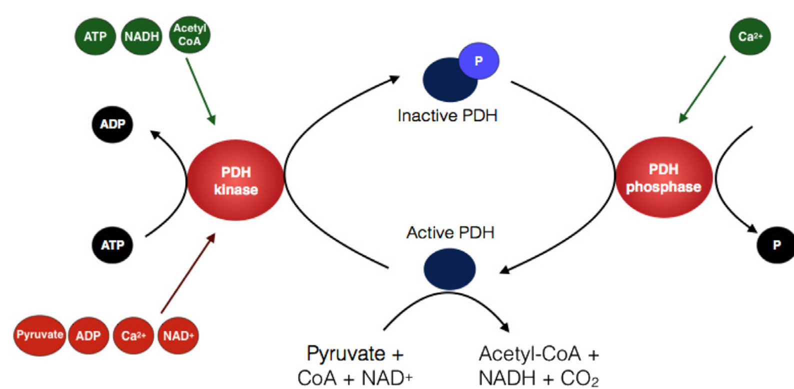

FIGURE 11.5 Regulation of PDH activity.
ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ca^{2+}, calcium; Pi, inorganic phosphate; PDH, pyruvate dehydrogenase.

(which exists in a dephosphorylated form due to the action of protein phosphatase 1). Given that phosphorylase can be transformed via covalent modification (i.e., phosphorylation by phosphorylase kinase) mediated through adrenaline, it would be reasonable to expect that greater phosphorylase transformation from *b* to *a* may be one mechanism to explain increased glycogenolysis evident with increasing exercise intensity. This would also be logical given that sarcoplasmic Ca^{2+} levels would be increased with high-intensity exercise (given the need for more rapid cross-bridge cycling) and that Ca^{2+} is a potent positive allosteric regulator of phosphorylase kinase through binding to the calmodulin subunit. However, the percentage of phosphorylase in the more active *a* form does not appear to be increased with exercise intensity and, in fact, is decreased after only 10 min of high-intensity exercise, which may be related to the reduced pH associated with intense exercise (Howlett et al., 1998). Whereas this mechanism of transformation (mediated by Ca^{2+} signaling) may be in operation within seconds of the onset of contraction (Parolin et al., 1999), it appears that *post transformational* mechanisms are in operation during more prolonged periods of high-intensity exercise given that glycogenolysis still occurs despite reduced transformation. In this regard, vital signals related to the energy status of the cell play a more prominent role. Indeed, as exercise intensity progresses from moderate to high-intensity exercise, the rate of ATP hydrolysis increases so much so that there is a greater accumulation of ADP, AMP, and Pi. In this way, the increased accumulation of Pi as a result of increased ATP hydrolysis can increase glycogenolysis as it provides increased substrate required for the reaction. Furthermore, greater accumulations of free ADP and AMP can also subsequently fine tune the activity of phosphorylase *a* through allosteric regulation (Howlett et al., 1998). Finally, although it is well documented that phosphorylase is under the hormonal control of adrenaline, infusion of adrenaline to levels beyond that of endogenous production during high-intensity exercise (85% $\dot{V}O_{2max}$) does not augment

glycogenolysis (Chesley et al., 1995), likely due to already sufficient activation of phosphorylase through the local mechanisms discussed earlier.

In addition to muscle glycogen, the contribution of plasma glucose to ATP production also increases with exercise intensity. The most likely explanation for this is due to increased muscle blood flow (and hence substrate delivery) in addition to increased muscle fiber recruitment (Rose and Richter, 2005). Although glucose uptake is also regulated by GLUT4 content, GLUT4 is unlikely to play a role in this situation given that GLUT4 translocation to the plasma membrane is not increased with exercise intensity (Kraniou et al., 2006). Once glucose is transported into the cytosol, it is phosphorylated to glucose-6-phosphate under the control of hexokinase. Evidence suggested that hexokinase activity is also not limiting given that patients with type 2 diabetes (who have reduced maximal hexokinase activity) display normal patterns of exercise-induced glucose uptake likely due to normal perfusion and GLUT4 translocation (Martin et al., 1995). In contrast, during intense exercise at near maximal or supra-maximal intensity, glucose phosphorylation may be rate limiting to glucose utilization given that high rates of glucose-6-phosphate secondary to muscle glycogen breakdown can directly inhibit hexokinase activity (Katz et al., 1986). Once glucose enters the glycolytic pathway, the rate-limiting enzyme to glycolysis is considered as phosphofructokinase (PFK). PFK is allosterically activated by ADP, AMP, and Pi and this mechanism is likely to explain high rates of glycolysis during intense exercise even in the face of metabolic acidosis when PFK could be inhibited.

In contrast to exercise intensity, prolonged steady-state exercise lasting several hours is characterized by a shift toward increased lipid oxidation and reduced CHO oxidation rates. This shift in oxidation rates is accompanied by an increased contribution of plasma FFA toward energy expenditure and a decreased reliance on both muscle glycogen and IMTGs. Studies examining the regulatory mechanisms underpinning this shift in substrate utilization have suggested that a reduction in muscle glycogen availability (due to progressive glycogen depletion) and hence a reduced glycolytic flux down-regulate PDH activity thereby leading to reduced CHO oxidation. In addition, progressive increases in plasma FFA availability (due to continual lipolysis in adipose tissue) stimulate lipid oxidation. The down-regulation of PDH activity as exercise duration progresses may be due to reduced pyruvate flux therefore reducing substrate production required for the PDH reaction (Watt et al., 2002). In addition, more recent data demonstrate an up-regulation of PDH kinase activity during exercise which would therefore directly inhibit PDH activity (Watt et al., 2004). Taken together, these data are consistent with the many observations that increasing or decreasing substrate availability is one of the most potent regulators of fuel utilization patterns during exercise and this concept is discussed in the next section.

11.3.2 Effects of Substrate Availability

Modifying substrate availability through dietary manipulation (such as loading regimens, preexercise meals or providing enhanced substrate availability during exercise) has been consistently shown to alter metabolic regulation during endurance exercise through various control points. Increasing muscle glycogen concentration enhances glycogenolysis during exercise (Hargreaves et al., 1995) by enhancing phosphorylase activity given that glycogen is a substrate for phosphorylase. The enhanced glycogenolysis with elevated glycogen stores does not appear to affect muscle glucose uptake (Hargreaves et al., 1995; Arkinstall et al., 2004). In addition to glycogenolysis, muscle glycogen also appears to be a potent regulator of PDH activity (and thus CHO oxidation) during exercise. Indeed, commencing exercise with reduced muscle glycogen attenuates the exercise-induced increase in PDH activity and vice versa (Kiilerich et al., 2010), likely due to reduced glycolytic flux as well as increased resting content of PDK4 (the kinase responsible for deactivating PDH) when glycogen concentration is low. PDH regulation appears particularly sensitive to nutritional status even at rest. In fact, just three days of a low CHO (but increased fat diet) up-regulates PDH kinase activity and down-regulates PDH activity (Peters et al., 1998).

Although the effects of exercise intensity on substrate utilization were discussed previously, it appears that muscle glycogen availability can influence fuel metabolism over and above that of exercise intensity. Indeed, Arkinstall et al. (2004) observed that glycogen utilization was enhanced during exercise at 45% $\dot{V}O_{2_{max}}$ that was commenced with high glycogen ($591\ \text{mmol} \cdot (\text{kg d.w.})^{-1}$) as opposed to exercise at 70% $\dot{V}O_{2_{max}}$ commenced with low glycogen concentration ($223\ \text{mmol} \cdot (\text{kg d.w.})^{-1}$) despite the higher intensity. In contrast to glycogen utilization and CHO oxidation rates, lipid oxidation was highest when exercise was commenced with reduced glycogen stores. The shift towards fat oxidation when preexercise muscle glycogen is low is likely mediated by a number of contributing factors. Firstly, reduced glycogen availability is associated with increased plasma FFA availability as well as adrenaline concentrations thus favoring conditions for augmented lipid oxidation and lipolysis, respectively, compared with conditions of high glycogen concentration (Arkinstall et al., 2004). However, when a preexercise meal is ingested and glucose infused during glycogen-depleted exercise such that minimal differences exist between plasma FFA and adrenaline, lipid oxidation is still augmented (Roepstorff et al., 2005). In such circumstances, available evidence points to regulation within the

muscle cell itself and more specifically, a carnitine mediated increase in lipid oxidation. Indeed, these researchers observed lower PDH activity, acetyl-CoA, and acetyl carnitine content and increased free carnitine concentrations during exercise when glycogen depleted compared with glycogen loaded conditions. Interestingly, ACC phosphorylation increased and malonyl CoA decreased similarly in both conditions despite higher AMPK activity when glycogen was reduced. Such data provide further evidence that malonyl CoA is not involved in regulating lipid metabolism during exercise but provide further support for a critical role of carnitine in regulating the interaction between CHO and lipid utilization (Wall et al., 2011).

When compared with exercise after overnight fasting, ingestion of CHO-rich meals within the hours before exercise (as well as CHO ingestion during exercise) has been shown to enhance endurance performance (Wright et al., 1991). Consequently, it is common practice for athletes to adopt such dietary approaches to competition. However, it is now well documented that pre- and during exercise CHO ingestion is one of the most potent ways to alter the pattern of CHO utilization during exercise through a number of control points. One of the main responses to CHO feeding is to attenuate plasma FFA availability and lipid oxidation while simultaneously increasing CHO oxidation rates. The reduced plasma FFA availability is due to an attenuation of lipolysis that is regulated by increased circulating insulin concentrations caused by CHO feeding. The antilipolytic effect of insulin is mediated through its ability to activate the enzyme phosphodiesterase which degrades cAMP and thereby attenuates activation of protein kinase A and eventually hormone sensitive lipase (HSL).

Convincing data confirming that lipolysis limits fat oxidation following CHO feeding is provided by Horowitz et al. (1997). In this study, male subjects completed 60 min of exercise at 45% $\dot{V}O_{2_{max}}$ in fasted conditions or 1 h after consuming 0.8 $g \cdot kg^{-1}$ of glucose (to induce a high insulin response), 0.8 $g \cdot kg^{-1}$ fructose (to induce a low insulin response) or an additional glucose trial during which intralipid and heparin were infused so as to maintain plasma FFA availability in the face of high insulin. In accordance with the insulin response, lipolysis (as indicated by rate of appearance of glycerol) was reduced with CHO feeding and plasma FFA availability was reduced in these conditions. In addition, rates of lipolysis exceeded lipid oxidation rates during fasted exercise, whereas in the CHO conditions, rates of lipolysis appeared to equal lipid oxidation rates thus implying that lipolysis limits fat oxidation. However, when intralipid and heparin was infused during an additional glucose trial, lipid oxidation rates were enhanced by 30% (4.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) compared with the glucose only trial (3.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) but were still not restored to levels occurring during fasted exercise (6.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Taken together, while these data suggest that only small elevations in insulin can attenuate lipolysis (i.e., 10−30 μU/mL), they also demonstrate a limitation within the muscle cell itself during CHO fed conditions. In accordance with reduced lipid oxidation following CHO feeding, CHO oxidation was increased due to increased glucose uptake (and oxidation) as well as muscle glycogenolysis. The enhanced rates of glycogenolysis was suggested to be due to increased allosteric activation of phosphorylase given that AMP and Pi production is greater during conditions of reduced plasma FFA availability, as is the case with CHO feeding.

In an effort to ascertain the source of limitation to lipid oxidation within the muscle following CHO feeding, Coyle et al. (1997) infused octanoate (an MCFA) or palmitate (an LCFA) during 40 min of exercise at 50% $\dot{V}O_{2_{max}}$ after an overnight fast or 60 min after ingesting 1.4 $g \cdot kg^{-1}$ of glucose. As expected (based on the previously discussed study), plasma FFA and lipid oxidation was higher in the fasted trials while CHO oxidation was lower in this condition compared with the glucose trials. However, the major finding of this study was that the percentage of palmitate oxidized during the glucose trial was reduced compared with fasting (70% vs 86%, respectively) whereas octanoate was unaffected (99% vs 98%, respectively). These data therefore suggest that LCFA uptake into the mitochondria is reduced with CHO feeding and when taken in the context of previous sections in this chapter, it becomes increasingly apparent that any condition which accelerates glycolytic flux (e.g., increased intensity, muscle glycogen, glucose feeding) can regulate intramuscular lipid metabolism, which again points to a carnitine mediated limitation. Furthermore, more recent data has demonstrated that the increased insulin and decreased adrenaline levels which accompany glucose ingestion during exercise appears to result in an attenuation of intramuscular HSL activity (Watt et al., 2004), thus highlighting an additional point of control.

11.3.3 Effects of Training Status

Endurance training results in a number of profound physiological and metabolic adaptations which function to reduce the degree of perturbations to homeostasis for a given exercise intensity and ultimately, delay the onset of fatigue. Adaptations to endurance training are most recognized functionally by an increase in maximal oxygen uptake as well as a rightward shift in the lactate threshold. From a metabolic perspective, the most prominent adaptation is an increase in the size and number of mitochondria (i.e., mitochondrial biogenesis) which essentially permits a closer matching between ATP requirements and production via oxidative metabolism. The adaptive response of

muscle mitochondria is also accompanied by increases in capillary density, substrate transport proteins and increased activity of the enzymes involved in the main metabolic pathways. In addition, endurance training increases the capacity for skeletal muscle to store glycogen and triglycerides thereby increasing substrate availability. In relation to substrate utilization during exercise following endurance training, the most notable response is a reduction in CHO utilization with a concomitant increase in lipid oxidation (Henriksson, 1977).

For a given exercise intensity, glycogen utilization is reduced with exercise training (Karlsson and Saltin, 1971), an effect that is confined locally to the actual muscles that were trained (Saltin et al., 1976). The reduced glycogenolysis observed after training was not due to any change in phosphorylation transformation, but rather allosteric mechanisms (Chesley et al., 1996; Le Blanc et al., 2004). Indeed, exercise in the trained state is associated with reduced content of ADP, AMP, and Pi thereby providing a mechanism leading to reduced phosphorylase activity. Le Blanc et al. (2004) also observed reduced pyruvate and lactate production during exercise undertaken in the trained state as well as reduced PDH activity. As a result of the reduced CHO flux, it is therefore likely that the attenuated pyruvate production (in addition to reduced ADP accumulation) may have attenuated PDH activity.

In addition to training-induced reductions in muscle glycogenolysis, several investigators have observed that training reduces exercise-induced liver glycogenolysis, as demonstrated by the rate of appearance of glucose in the circulation. There is some evidence (although this is not consistent within the literature) that endurance training also increases skeletal muscle gluconeogenesis following training (Bergman et al., 2000). In accordance with reduced rates of glucose production, muscle glucose uptake is reduced when exercise is undertaken at the same absolute workload following a period of endurance training (Bergman et al., 1999).

Despite the fact that training increases total muscle GLUT4, the reduction in exercise-induced muscle glucose uptake is most likely caused by a reduced *translocation* of GLUT4 to the sarcolemma following training thereby reducing the capacity to transport glucose (Richter et al., 1998). This particular study utilized a knee extensor training and exercise model where only one limb was trained but yet both limbs performed the exercise protocol before and after training. In this way, training-induced alterations in hormonal and cardiovascular status were minimized and the reduced glucose uptake and GLUT4 translocation was likely mediated by local contractile factors. In summarizing the link between liver glucose production and muscle glucose uptake, it is generally accepted that training-induced changes in hormone concentrations such as adrenaline, insulin, and glucagon are unable to explain all of the effects (Phillips et al., 1996). Rather, it is possible that the actual rate of muscle glucose uptake acts as a feedback signal to regulate glucose output from the liver (Phillips et al., 1996).

11.4 CARBOHYDRATE AND EXERCISE PERFORMANCE

Given the effects of exercise intensity, duration and training status on muscle glycogen utilization, it follows that glycogen depletion (in both muscle and liver) is a major cause of fatigue in both endurance and high-intensity (intermittent) type activities. As such, traditional nutritional advice for these types of activities (whether it is competitive situations or training sessions) is to ensure high daily CHO intake before, during and after the activity so as to promote both performance and recovery.

11.4.1 Muscle Glycogen and Carbohydrate Loading

The basic principles of CHO loading were developed in the late 1960s where it was identified that a period of exhaustive exercise followed by several days of high dietary CHO intake induces a super-compensation effect so that glycogen storage is augmented (Bergstrom and Hultman, 1966; Bergtrom et al., 1967). A less extreme form of CHO loading was developed in the 1980s where Sherman et al. (1981) observed that a simple exercise taper in conjunction with several days of increased dietary CHO intake was also sufficient to increase glycogen storage. It is now generally accepted that trained athletes can increase glycogen storage in both type I and II fibers within 24–48 h of increased CHO intake (Bussau et al., 2002). In relation to practical application, it is also accepted that high glycemic foods are superior to low glycemic foods (Burke et al., 1993) in augmenting glycogen storage and that dietary intakes of $8-12\ g \cdot kg^{-1}$ per day are required (Thomas et al., 2016). The general consensus from the wealth of studies undertaken in the past 40 years is that CHO loading can improve performance and capacity especially when the exercise is greater than 90 min in duration (Hawley et al., 1997). The enhanced performance effect is likely *initially* mediated by a delay in the time-point at which energy availability becomes limiting to the maintenance of the desired workload, which in the case of *race pace* is dependent on sustained and high rates of CHO oxidation (O'Brien et al., 1993; Leckey et al., 2016). Indeed, in reviewing the literature Hawley et al. (1997) cited that CHO loading can improve exercise

capacity by approximately 20% and time-trial performance can increase by 2%−3%. In addition to providing substrate availability for ATP production, it is now recognized that glycogen availability (especially the intramyofibrillar storage pool) can directly modulate contractile function. Indeed, a series of studies from Ørtenblad et al. (Ørtenblad et al., 2011, 2013; Geji et al., 2014) have collectively shown a preferential utilization of this storage pool during exercise in a manner that also correlates with impaired Ca^{2+} release from the SR. Such impaired excitation-contraction (EC) coupling is likely to be of particular importance during those situations where higher power outputs and sprint finishes are required in the very late and finishing stages of races.

11.4.2 Preexercise Carbohydrate Availability

Whereas the 1960s and 1970s focused on CHO loading studies, research in the next two decades examined the effects of preexercise feeding as well as consuming additional CHO during exercise. Preexercise feeding (i.e., 3−4 h before competition) is not only advantageous as it can lead to further elevations in muscle glycogen content (Wee et al., 2005) but can also restore liver glycogen content which is usually depleted after an overnight fast. The latter is particularly important given that liver glycogen content is related to exercise capacity (Casey et al., 2000). Sherman et al. (1991) observed that time-trial performance after 90 min of steady-state exercise at 70% $\dot{V}O_{2_{max}}$ was greater when 150 g of CHO was consumed before exercise compared with 75 g of CHO, both of which were greater than no meal. The enhanced performance effect was associated with maintenance of blood glucose concentration late during exercise which is important because liver glucose production and muscle glucose uptake and oxidation become more important when muscle glycogen concentrations begin to decline. In a further study, the same authors also observed that performance can be further increased when CHO is ingested during exercise in addition to a preexercise meal (Wright et al., 1991). As such, current CHO guidelines for preexercise feeding advise an intake of $1-4\ g \cdot kg^{-1}$ body mass 3−4 h prior to exercise (Thomas et al., 2016).

11.4.3 Carbohydrate Feeding During exercise

In addition to high endogenous preexercise muscle glycogen stores, it is widely accepted that exogenous CHO feeding during exercise also improves physical, cognitive and technical elements of performance (Stellingwerff and Cox, 2014). Whereas it was generally accepted that exogenous CHO oxidation rates were thought to be limited at approximately $1\ g \cdot min^{-1}$ due to saturation of intestinal glucose transporters, it is now known that exogenous CHO oxidation rates can increase to $1.8\ g \cdot min^{-1}$ with the addition of sucrose or fructose to the CHO blend (Jeukendrup, 2014). When taken together, it is thought that CHO feeding during exercise may therefore augment exercise performance via multiple mechanisms consisting of muscle glycogen sparing (Stellingwerff et al., 2007), liver glycogen sparing (Gonzalez et al., 2015) and maintenance of plasma glucose and CHO oxidation rates (Coyle et al., 1986). However, despite the proposed mechanisms it is important to acknowledge that exercise duration, intensity, nutritional status prior to exercise, CHO intake rate and the CHO type/blend consumed during exercise will all have an impact upon the efficacy of these mechanisms, fuel metabolism and performance.

It is noteworthy that exogenous CHO feeding during exercise also improves performance (Jeukendrup et al., 1997) when exercise duration is <60 min (i.e., glycogen availability is not limiting), an effect that is not apparent when glucose is directly infused to the bloodstream during exercise (Carter et al., 2004a). Such data suggest that CHO feeding may also improve exercise performance via nonmetabolic effects but through direct effects on the central nervous system (Carter et al., 2004b). To this end, the last decade of research has resulted in a growing body of literature demonstrating that simply "rinsing" CHO in the oral cavity (for 10-s periods every 5−10 min during exercise) is also ergogenic to performance (Burke and Maughan, 2015), an effect that is independent of sweetness (Chambers et al., 2009) and that is especially apparent in the absence of a preexercise CHO meal (Lane et al., 2013) and low preexercise muscle glycogen (Kasper et al., 2015).

The conventional approach to CHO fueling during exercise is to consume 6%−8% CHO beverages, although relying solely on this approach does not allow for flexibility in terms of individual variations in body mass or actual fluid requirements given variations in ambient conditions (Lee et al., 2014). As such, many athletes rely on a CHO fueling approach that is based on a combination of solids (e.g., bars), semi-solids (e.g., gels) and fluids (e.g., sports drinks) so as to collectively meet their personalized exogenous CHO targets, typically in the region of $30-90\ g \cdot h^{-1}$ depending on exercise duration. Nevertheless, although there is little difference in exogenous CHO oxidation rates (albeit in fluid matched conditions) between the aforementioned sources (Pfeiffer et al., 2010a,b), it is noteworthy that many athletes experience gastrointestinal discomfort when attempting to hit these targets, possibly related to extreme differences in osmolality between commercially available CHO gels (Zhang et al., 2015) as well as the presence of fiber, fat, and protein in energy bars (Pfeiffer et al., 2012). As such, it is now advised that athletes should clearly practice their

approach to in-competition fueling during those training sessions of similar intensity and duration as competition. As a general rule of thumb, it is suggested that $30-60\ g \cdot h^{-1}$ of CHO (glucose polymers) is consumed during events lasting $<60-90$ min whereas in events $>2-3$ h, $60-90\ g \cdot h^{-1}$ (glucose/fructose blends) is the recommended rate (Thomas et al., 2016)

11.5 CARBOHYDRATE AND TRAINING ADAPTATION

11.5.1 Overview of Molecular Regulation of Training Adaptations

Being a highly malleable tissue, skeletal muscle has the ability to undergo major adaptations and alter its phenotype in response to exercise stimuli. Upon the onset of muscle contraction, multiple molecular signaling pathways are activated which subsequently contribute to the adaptive responses within skeletal muscle. In the past decade of research, accumulating data suggest that these signaling pathways are also sensitive to nutrients as well as exercise, and that this cross-talk between exercise and nutrition stimuli can be manipulated to up-regulate the adaptive responses to training. Endurance athletes typically focus their training to enhance those adaptations within the muscle which will subsequently increase exercise capacity and fatigue resistance. Such adaptations include enhanced cardiac output, increased mitochondrial content, lipid oxidation and angiogenesis, all of which are recognized functionally by increased whole-body oxygen uptake ($\dot{V}O_{2_{max}}$) and rightward shift of the lactate threshold curve. From an endurance perspective, perhaps the most important of these adaptive responses is the increase in mitochondrial content, termed *mitochondrial biogenesis*. This increase in mitochondrial mass ultimately allows endurance athletes to exercise at higher intensities for longer periods. A schematic overview of the proposed mechanisms underpinning skeletal muscle adaptive responses to training is displayed in Fig. 11.6.

In response to each individual exercise bout, acute transcriptional changes take place within the muscle in the hours during recovery, and it is the accumulation of these acute responses over time that subsequently alters the muscle to a more oxidative phenotype through the expression of new proteins (Perry et al., 2010). When muscle contraction begins, a number of metabolic perturbations within muscle cells (i.e., increased AMP/ATP ratio, Ca^{2+} flux, lactate, hypoxia, and energy availability) occur which collectively trigger the activation of key regulatory protein kinases. The most extensively studied of these kinases are p38 mitogen-activated protein kinase (p38MAPK), AMP-activated protein kinase (AMPK), sirtuin 1 (SIRT1), and calmodulin-dependent protein kinase II (CaMKII). These kinases subsequently phosphorylate downstream targets such as transcription factors and transcriptional coactivators to induce the up-regulation of gene expression (Jager et al., 2007). The most well-studied regulator of mitochondrial biogenesis is the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). The importance of PGC-1α as a mediator of mitochondrial biogenesis is evident from rodent studies demonstrating that overexpression increases oxidative enzyme activity (Lin et al., 2002) and improves exercise capacity (Calvo et al., 2008). In humans, elevated PGC-1α mRNA levels are observed following endurance exercise with the highest abundance typically present in the first 2$-$4 h of recovery (Gibala et al., 2009; Bartlett et al., 2012).

Both AMPK and p38MAPK can directly phosphorylate PGC-1α during acute exercise resulting in its translocation to both the nucleus and the mitochondria. In the nucleus, it interacts with transcription factors such as NRF-1, NRF-2, estrogen-related receptor (ERRα), peroxisome proliferator-activated receptor (PPARδ), and myocyte enhancer factor 2 (MEF2), to induce the up-regulation of proteins involved in glucose and fatty acid transport and oxidation. Upon localization to the mitochondria, PGC-1α forms a complex with mitochondrial transcription factor A (Tfam) at the D-loop region to coordinate up-regulation of muscle mitochondrial content and the capacity for substrate metabolism and oxidative phosphorylation (Safdar et al., 2011). In addition to PGC-1α, the tumor suppressor protein p53 has now emerged as a potential regulator of mitochondrial biogenesis. Indeed, acute exercise induces the posttranslational modification of p53 (Bartlett et al., 2012), and similarly to PGC-1α, this protein also translocates to the nucleus (Philip et al., 2011) and the mitochondria (Saleem and Hood 2013) to interact with Tfam.

The principle of promoting high CHO availability before, during, and after exercise is the foundation on which traditional sports nutrition guidelines are based. Although this is essential for promoting maximal competition performance and ensuring adequate recovery, accumulating data now suggest that restricting CHO before, during, and in recovery from endurance-based exercise augments the cell signaling and gene expression responses associated with oxidative adaptations in human skeletal muscle. Indeed, both acute and training-based studies have collectively observed that the reduction of both endogenous and/or exogenous CHO promotes mitochondrial enzyme activity and protein content, increases both whole body (Yeo et al., 2008b) and intramuscular Hulston et al. (2010) lipid metabolism and can improve both exercise capacity (Hansen et al., 2005) and performance (Marquet et al., 2016). This approach to CHO periodization has been termed *train-low, compete-high*, a model

which promotes CHO restricted training for augmenting adaptation, but ensures high CHO availability during competition to promote maximal performance.

11.5.2 Fasted Training

The idea that CHO restriction augments markers of training adaptation first emerged when data demonstrated enhanced expression of genes involved in mitochondrial biogenesis and substrate oxidation following exercise undertaken with reduced muscle glycogen availability. For example, Pilegaard et al. (2002) demonstrated that the acute exercise induced increases in PDK4, UCP3, and CPT1 mRNA expression were all augmented to a greater extent when preexercise muscle glycogen levels were low compared to normal levels. Similarly, both Cluberton et al. (2005) and Civitarese et al. (2005) observed increases in those genes involved in metabolic regulation when commencing exercise following an overnight fast rather than the ingestion of a CHO-rich breakfast. Data from the latter study also suggest that the expression of genes involved in the regulation of lipid metabolism was suppressed when CHO was fed before, during, and after 2-h cycling as opposed to the same exercise undertaken in the fasted state. In a subsequent 6-week training study design, Van Proeyen et al. (2011) attempted to elucidate whether these responses to a single bout of fasted exercise could be sustained over a longer period. The authors observed augmented citrate synthase (CS) and β-HAD activity when regular 2-h steady-state cycling was performed in the fasted state compared to following the consumption of a breakfast. Nonetheless, the augmented biochemical adaptations did not translate to improved exercise performance.

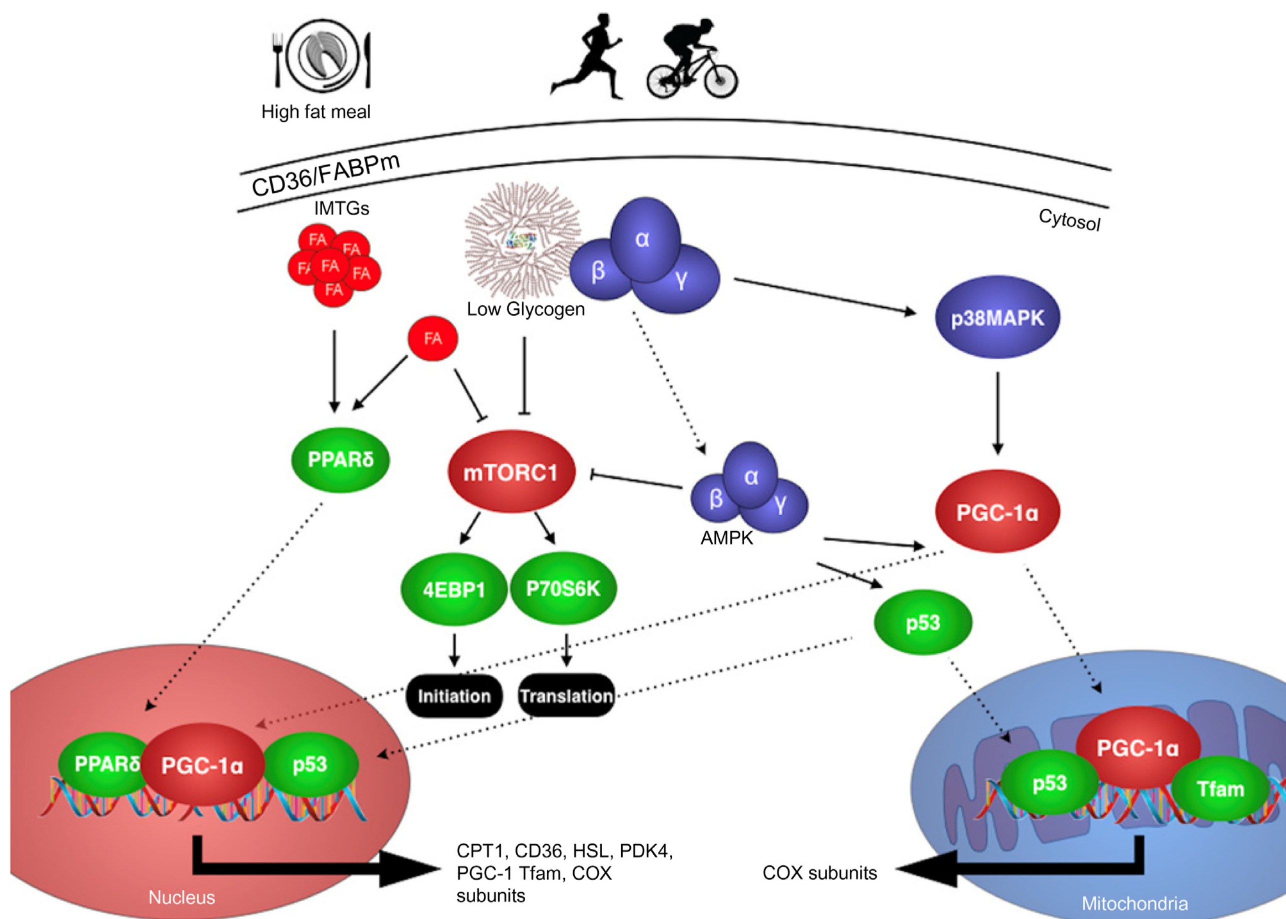

FIGURE 11.6 Overview of key molecular signaling pathways regulating endurance training adaptations.
4EBP1, eukaryotic translation initiation factor 4E-binding protein 1; AMPK, 5′ adenosine monophosphate-activated protein kinase; COX, cytochrome c oxidase; CPT-1, carnitine palmitoyltransferase 1; FA, fatty acid; FABP, fatty acid binding protein; HSL, hormone sensitive lipase; IMTGs, intramuscular triglycerides; mTORC1, mammalian target of rapamycin complex 1; p38MAPK, p38 mitogen-activated protein kinase; p70S6K, ribosomal protein S6 kinase beta-1; PDK4, pyruvate dehydrogenase lipoamide kinase 4; PPAR, peroxisome proliferator-activated receptor; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; Tfam, transcription factor A.

11.5.3 Postexercise Carbohydrate Restriction

In addition to restricting CHO prior to endurance exercise training, data also demonstrate beneficial adaptive responses when restricting CHO during the postexercise recovery period. Indeed, Pilegaard et al. (2005) explored this idea with participants completing 75-min of cycling at 75% $\dot{V}O_{2_{max}}$ followed by the consumption of a diet either high or low in CHO for the next 24-h. These authors observed that although the mRNA expression of PDK4, LPL, UCP3, and CPT1 increased in response to exercise, activation was only sustained in the low CHO group following the 24-h. In a twice-per-day, 6-week training study it has also been observed that when glucose is consumed during recovery from the first session, the enhanced oxidative adaptations are blunted compared to when CHO is restricted, despite reduced levels of muscle glycogen (Morton et al., 2009). When taken together, responses from these studies suggest that reducing CHO availability in the recovery period also modulates the muscle adaptive process.

11.5.4 Twice-per-day Training Models

On the basis of the molecular evidence derived from acute studies, Hansen et al. (2005) were the first to investigate the idea that repeated exercise (i.e., exercise training) with reduced CHO availability augments oxidative adaptations and subsequent endurance performance. In a 10-week long training study using single leg knee extensor exercise and training 5 days per week, participants either trained one limb every day with normal levels of muscle glycogen, or the contralateral limb twice every second day whereby the second session was undertaken with reduced muscle glycogen availability. Exercise during the twice-per-day sessions was interspersed with 2-h of recovery, during which time no CHO was consumed. In this way, both limbs performed identical work, but one limb performed 50% of these sessions with low muscle glycogen availability. The authors observed greater increases in CS activity in the limb which had undertaken training with lower levels of muscle glycogen compared to normal. Additionally, greater improvements in exercise capacity were observed in the "low" limb compared to normal, suggesting that repeatedly training in this way may lead to performance gains in the long term.

Yeo et al. (2008b) subsequently explored this concept using a "real-world" design more applicable to elite athletes. Using well trained male cyclists in a 3-week training block, cyclists trained 6 times per week, either once every day with high muscle glycogen availability in one group, or twice every other day so the second session was undertaken with reduced levels of muscle glycogen in the other group. In the "high" group cyclists alternated between steady state and HIT exercise each day, whereas in the "low" group, steady-state exercise was performed in the morning and HIT exercise performed after a 1−2-h recovery period during which time CHO was restricted. Before and after this training block, muscle biopsies were obtained to assess markers of adaptation, and a time-trial was completed to examine performance improvements in each group. Despite significant increases in CS and, β-HAD activity, COXIV protein content, and rates of fat oxidation in the "low" group following training, time-trial performance was still similar in both groups. Interestingly, the enhanced adaptive responses still occurred in the "low" group despite cyclists having to reduce exercise intensity during HIT training session. These findings suggest that even when overall training intensity is reduced, reduced CHO availability is associated with an adaptive response. In a similar study design, Hulston et al. (2011) also reported greater increases in intramuscular lipid oxidation and the expression of CD36 and β-HAD following training low compared with training high.

11.5.5 Sleep-Low/Train-Low Models

Subsequent train-low investigations have adopted a "sleep-low/train-low" approach, whereby participants perform an evening training session and then restrict CHO during the recovery period so they go to sleep with low levels of muscle glycogen. A morning training session is then subsequently performed the following day under levels of low muscle glycogen availability. This method was first examined using whole-body exercise by Bartlett et al. (2013), whereby participants were required to perform an acute bout of HIT running under conditions of either high or low CHO availability. In the low condition, participants had performed glycogen-depleting exercise the evening prior to the trial, and CHO was restricted during and in recovery from exercise. The phosphorylation of p53 was significantly higher immediately post and 3-h postexercise in the low compared to the high trial. Additionally, the mRNA expression of PDK4, Tfam, COXIV, and PGC-1α were all significantly greater in the low trial at 3-h postexercise compared to when CHO was consumed before, during, and after exercise. In a subsequent study (Lane et al., 2015), a sleep-low strategy was employed whereby participants ingested isoenergetic diets containing $8\ g \cdot kg^{-1}$ CHO, but timing of ingestion was altered to elicit sleeping low. They consumed either $8\ g \cdot kg^{-1}$ CHO prior to evening HIT then slept low, or consumed $4\ g \cdot kg^{-1}$ CHO prior to the evening HIT then $4\ g \cdot kg^{-1}$ CHO before bed. The following morning they then completed a 2-h steady-state cycling protocol. While fat oxidation and PDK4 mRNA expression were significantly greater following fasted morning exercise, those genes involved in the regulation of mitochondrial

biogenesis showed similar exercise induced increases in both groups (Lane et al., 2015). Since the participants in this study were highly trained, they still maintained high levels of muscle glycogen despite "sleeping low," thus further highlighting that the absolute muscle glycogen concentration may be an important regulatory factor in modulating adaptations, especially in well trained populations. More recent work from Marquet et al. (2016) focused on incorporating the sleep-low strategy as part of a 3-week training block with elite triathletes. Using a similar CHO feeding approach to Lane and colleagues, these authors observed that when the sleep-low training strategy was employed, 10km time-trial performance was improved significantly compared to when normal levels of CHO were consumed across the training block. When taken together, these findings collectively suggest that the sleep-low/train-low strategy is effective for not only further up-regulating the muscle adaptive responses to training, but also improving endurance performance.

While the mechanisms underpinning the aforementioned adaptive responses to both acute and chronic exercise are still not fully understood, they are likely mediated by upstream signaling from AMPK and p38MAPK. Indeed, AMPK has the capacity to be modulated by the glycogen status of the muscle through a glycogen-binding domain on the β-subunit (McBride et al., 2009), with data suggesting that AMPK is more active when glycogen is depleted. Findings from Wojtaszewski et al. (2003) indeed demonstrated that when preexercise muscle glycogen levels are reduced, AMPKα2 activity and ACC^{Ser221} phosphorylation are significantly elevated following steady-state cycling compared to when muscle glycogen is high. In a subsequent study, Chan et al. (2004) also observed a significantly greater nuclear abundance of p38MAPK both pre- and postexercise when muscle glycogen levels were low compared to high. In another twice-per-day train-low study, Cochran et al. (2010) also reported significantly greater elevations in p38MAPK phosphorylation following the second exercise session when participants consumed no CHO during recovery. These data are highly suggestive of both AMPK and p38MAPK being nutrient sensitive, and thus likely regulating the downstream events leading to increases in mitochondrial biogenesis including p53 and PGC-1α activation.

11.5.6 High-Fat Feeding

In addition to the manipulation of CHO availability to promote training adaptations, data also suggest a potential modulatory role of high-fat availability in augmenting the training response. Indeed, many studies have demonstrated shifts in substrate utilization during exercise following "fat adaptation" protocols, however, there still remains little evidence that this has any actual beneficial effect on performance (Burke, 2015). It is possible that acute elevations in circulating FFA availability during exercise may regulate key cell-signaling kinases and transcription factors as well as modulating the expression of genes regulating both CHO and lipid metabolism. Indeed, Zbinden-Foneca et al. (2013) observed suppressions in p38MAPK during exercise following the pharmacological ablation of FFA availability when compared with control conditions. Additionally, the enhanced p38MAPK phosphorylation observed by Cochran et al. (2010) using a twice-per-day exercise model was associated with enhanced circulating FFA availability during the afternoon exercise. When taken together these data suggest that FFAs may act as signaling intermediates for p38MAPK when CHO is low. Further studies have also observed increases in resting intramuscular triglyceride stores, HSL, AMPK-α2 activity (Yeo et al., 2008a), and increases in the protein content of CD36 (Cameron-Smith et al., 2003) in response to 5 days of high-fat feeding. Such adaptations undoubtedly contribute to the enhanced rates of lipid oxidation observed during exercise following "fat adaptation" protocols and would appear beneficial for endurance athletes.

Nonetheless, it is noteworthy that high-fat feeding may actually impair glycogen utilization during exercise. Indeed, Stellingwerff et al. (2006) observed a significant reduction in PDH activity following 5 days of high-fat feeding, likely inhibiting the entry of CHO in to the Krebs cycle. More recently, data also demonstrates that acute high-fat feeding significantly increases the mRNA expression of PDK4 for up to 15-h postexercise, indicative of suppressive effects on CHO metabolism through the PDH complex (Hammond et al., 2016). When taken together, these findings suggest that rather than preparing elite athletes for competition, high-fat feeding may actually negate the capacity to utilize CHO during high-intensity exercise thus impairing performance. Indeed, in a study examining the effects of a ketogenic diet during three weeks of intense training in elite race walkers, Burke et al. (2017) observed that despite improvements in whole-body oxidation rates, economy, and overall performance were negatively impacted by this type of feeding when compared with periodized high CHO availability.

Moreover, although many endurance training-induced skeletal muscle adaptations are regulated at a transcriptional level, the turnover of myofibrillar (i.e., contractile) proteins are largely regulated through the translational machinery and the mechanistic target of rapamycin complex (mTOR) and ribosomal protein S6 kinase 1 (p70S6K) signaling axis (outlined in Fig. 11.6) (Moore et al., 2014). In this regard, data collected from lipid and heparin infusion suggests that high circulating FFA availability actually impairs muscle protein synthesis

(Stephens et al., 2015). Hammond et al. (2016) investigated this concept using a twice-per-day, whole-body exercise model whereby participants completed morning HIT running and afternoon steady-state running (3.5-h recovery between sessions) under conditions of either high CHO, or low CHO but high-fat availability. Indeed, authors observed suppression in the activity of p70S6K at 3-h postexercise in the high-fat feeding trial, suggesting that postexercise high-fat feeding may actually impair skeletal muscle remodeling process. Additionally, it was also observed that the exercise induced increases in PGC-1α, p53, Tfam, CS, and ERRα were not different between trials, suggesting no additional benefit high-fat feeding during periods of train-low.

11.5.7 Muscle Glycogen Threshold

Although it is now accepted that muscle glycogen availability is a potent regulator of the adaptive responses of skeletal muscle to exercise training, the level of absolute glycogen required to augment the pathways regulating mitochondrial biogenesis is currently unknown. However, it appears that a "glycogen threshold" may exist, whereby a critical absolute level of glycogen must be exceeded in order for significant activation of specific cell-signaling pathways to occur. The majority of studies that adopt a low glycogen model commence exercise with glycogen concentrations between 100–300 mmol · (kg d.w.)$^{-1}$, where the activity of key cell-signaling kinases, transcription factors, and transcriptional coactivators and expression of various metabolic genes are augmented when compared with exercise commenced with high (350–900 mmol · (kg d.w.)$^{-1}$) glycogen (see Fig. 11.7). As such, it would appear important that exercise is commenced with muscle glycogen concentrations below 350 mmol · (kg d.w.)$^{-1}$ when undertaking a train-low exercise session. Nonetheless, it also appears that significant activation of cell-signaling pathways controlling mitochondrial biogenesis can still be achieved with high preexercise glycogen concentrations as long as a critical absolute amount of glycogen is exceeded during exercise (and some exercise is therefore performed under conditions of low glycogen). For instance, Impey et al. (2015) demonstrated that exhaustive exercise induces significant activation of AMPK and expression of transcription factors (p53, Tfam) and coactivators (PGC-1α), even when commenced with high glycogen levels (600 mmol · (kg d.w.)$^{-1}$). This is likely due to the fact that subjects surpassed a critical level of glycogen ($\sim$350 mmol · (kg d.w.)$^{-1}$) during exercise and reached exhaustion at very low levels ($\sim$100 mmol · (kg d.w.)$^{-1}$), therefore performing a significant proportion of exercise with low muscle glycogen. Although significant activation of cell-signaling cascades appears possible with high preexercise glycogen levels, what is clear is that significantly more "work" is required to achieve the same signaling effects, whereby commencing exercise with low glycogen induces "work-efficient" cell signaling related to mitochondrial biogenesis. For instance, the aforementioned work demonstrates that training with low preexercise ($\sim$300 mmol · (kg d.w.)$^{-1}$) glycogen induces a significant activation of AMPK in significantly less time ($\sim$60 min) than when training is commenced with high glycogen.

ACC, acetyl-CoA carboxylase; AMPK, 5′ adenosine monophosphate-activated protein kinase; Ca^{2+}, calcium; COX, cytochrome c oxidase; p38, p38 mitogen-activated protein kinase; p70S6K, ribosomal protein S6 kinase beta-1; PDK4, pyruvate dehydrogenase lipoamide kinase 4; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; Tfam, transcription factor A.

Further support for the notion of a glycogen threshold is also provided from studies that have fed CHO during exercise. Indeed, when glycogen utilization during exercise is attenuated through exogenous CHO supplementation (i.e., glycogen sparing) and hence does not surpass a "critical limit," AMPK activity is reduced (Akerstrom et al., 2006). Interestingly, CHO supplementation prevented muscle glycogen concentrations surpassing 300 mmol · (kg d.w.)$^{-1}$ (similar to that of the proposed "critical threshold"), whereas when glycogen was reduced to 200 mmol · (kg d.w.)$^{-1}$ in the placebo trial, a significant activation of AMPK occurred (Akerstrom et al., 2006). In contrast, when exogenous CHO supplementation does not spare muscle glycogen (and therefore allows depletion below a "critical limit") (< 200 mmol · (kg d.w.)$^{-1}$) AMPK activity is not suppressed (Lee-Young et al., 2006). While training with glycogen concentrations below a critical limit appears beneficial for the activation of cell-signaling cascades regulating mitochondrial biogenesis, it appears that keeping glycogen at these levels may impair the regulation of postexercise muscle protein synthesis. Indeed, subsequent work from Impey et al. (2016) demonstrates that p70S6K activity is suppressed when glycogen concentrations reach very low levels ($\sim$100 mmol · (kg d.w.)$^{-1}$) despite feeding leucine enriched whey protein. However, repletion of muscle glycogen to $\sim$250 mmol · (kg d.w.)$^{-1}$, via sufficient postexercise CHO provision, appears to re-activate p70S6K activity.

In addition to regulating cell-signaling pathways controlling mitochondrial biogenesis and muscle protein synthesis, muscle glycogen also appears to regulate SR calcium handling and thus skeletal muscle function, whereby contractile properties are impaired when glycogen concentrations fall below a critical limit. Chin and Allen (1997) elegantly demonstrated that when recovery from glycogen reducing contractions occurs in the absence of glucose and thus glycogen remains low, fiber bundles fatigue at a faster rate and show reduced tetanic Ca^{2+} transients in a subsequent fatigue test. These findings have been subsequently confirmed in human skeletal

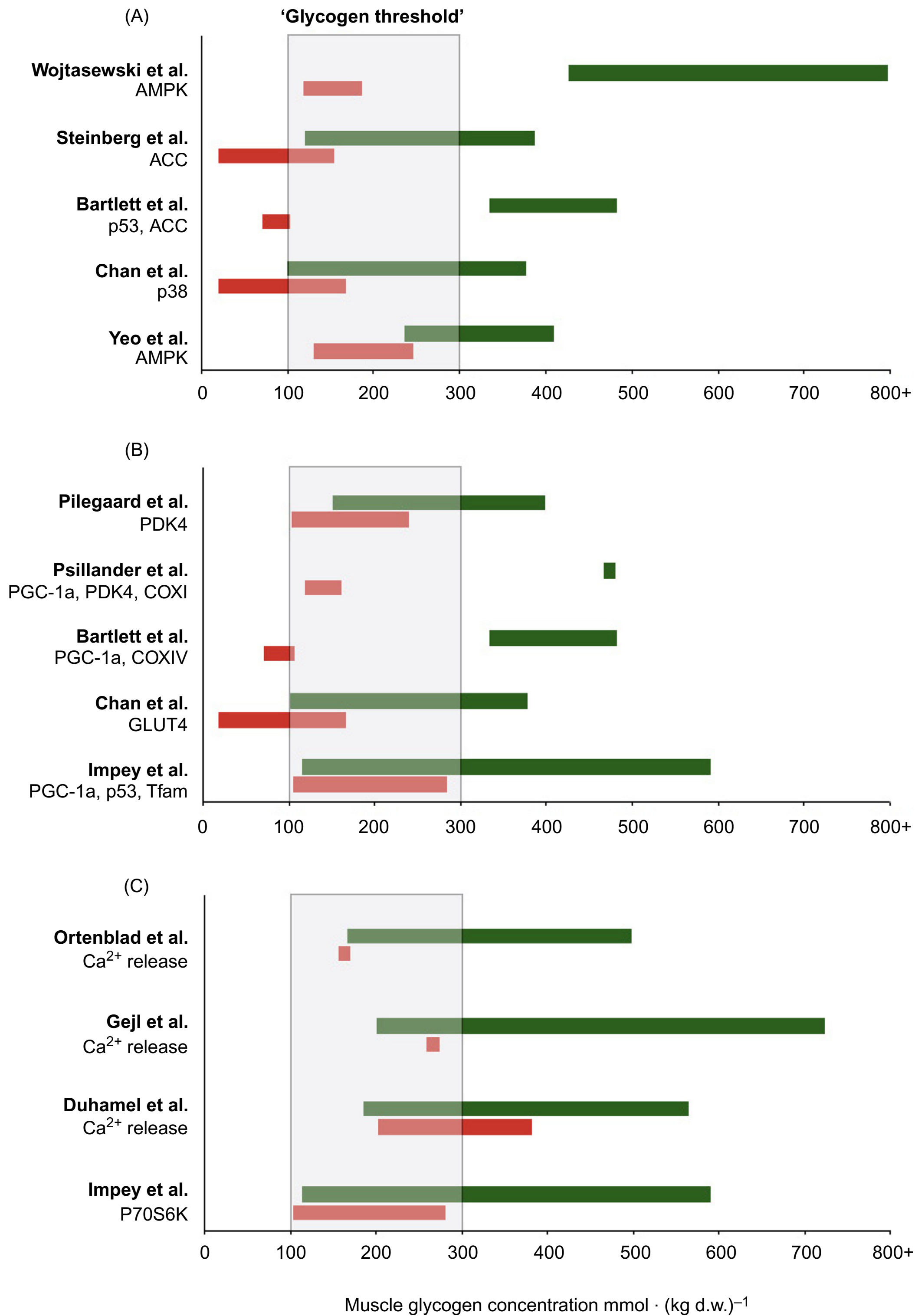

FIGURE 11.7 Summary of studies demonstrating differential metabolic responses of skeletal muscle in response to exercise commenced in conditions of high or low muscle glycogen availability. Studies are categorized into those examining a) cell signalling, b) gene expression and c) muscle contractile capacity and post-exercise signalling Shaded area represents proposed muscle glycogen threshold. Red bars represent low CHO trials and green bars represent high CHO trials. The width of the bar represents starting and end point of muscle glycogen during the relevant exercise trials. *Taken from Fig. 11.2, Impey, S.G., et al., 2018. Sports. Med, under the terms of the Creative Commons Attribution 4.0 International License, https://creativecommons.org/licenses/by/4.0/.*

muscle, where an impairment in SR Ca^{2+} release rate and subsequent power output are apparent under conditions of low muscle glycogen (Duhamel, Perco and Green, 2006; Ortenblad et al., 2011; Gejl et al., 2014). Intriguingly, this impairment appears to occur at muscle glycogen concentrations below 300 mmol · (kg d.w.)$^{-1}$, similar to the proposed critical threshold required to significantly activate cell-signaling pathways regulating mitochondrial biogenesis. Given the importance of Ca^{2+} for EC coupling and subsequent muscle contraction, it appears that low glycogen concentrations may inhibit the ability of skeletal muscle to contract and muscle fibers may fatigue more rapidly. When taken together, these data further allude to a potential muscle glycogen threshold, surmising that low muscle glycogen may not only enhance the activation of pathways regulating mitochondrial biogenesis, but also regulate skeletal muscle contractile properties and postexercise muscle protein synthesis if kept at critically low levels.

11.5.8 Practical Applications

Despite the clear rationale of the train-low paradigm, there are a number of potential limitations to this type of training that can make it difficult for exercise physiologists and nutritionists to best periodize this type of training in to an elite athlete's training schedule. Indeed, reduced CHO availability impairs acute training intensity (Yeo et al., 2008b; Hulston et al., 2011) and hence if performed long term, may actually lead to a de-training effect. Additionally, given the role of CHO in preventing immunosuppression, it is possible that repeated high-intensity training under conditions of low CHO increases susceptibility to illness and infection (Gleeson et al., 2004). Restriction of CHO availability has also been shown to increase muscle protein breakdown (Howarth et al., 2010), an effect that if performed chronically may lead to muscle mass loss especially in conditions both calorie and CHO restriction. Finally, data also demonstrate a reduced ability to oxidize exogenous CHO following regular training with low CHO, which could lead to a negative effect on competition performance (Cox et al., 2010). Taking the above limitations into account, it is important to recognize that training with low CHO availability should be carefully periodized in an athlete's training program.

In summary, this body of literature alludes to a potential muscle glycogen threshold (e.g., <350 but >150 mmol · (kg d.w.)$^{-1}$) surmising that reduced preexercise muscle glycogen may enhance the activation of those pathways regulating mitochondrial biogenesis but also suggest that keeping glycogen (and energy intake) at critically low levels (i.e., <100 mmol · (kg d.w.)$^{-1}$) may impair the regulation of postexercise remodeling processes. In practice, this approach could represent an amalgamation of train-low paradigms and is perhaps best communicated by the principle of "fuel for the work required". Indeed, athletes could strategically reduce CHO availability prior to completing predetermined training workloads that can be readily performed with reduced CHO availability, thereby inducing a "work-efficient" approach to training. Alternatively, when the goals of the training session are to complete the highest workload possible over more prolonged durations, then adequate CHO should be provided in the 24 h period prior to and during the specific training session. Careful day-to-day periodization in a meal-by-meal manner (as opposed to chronic periods of CHO restriction) is likely to maintain metabolic flexibility and still allow for the completion of high-intensity and prolonged duration workloads on heavy training days e.g., interval type workouts undertaken above lactate threshold. Intuitively, train-low sessions may be best left to those training sessions that are not CHO dependent and in which the intensity and duration of the session is not likely to be compromised by reduced CHO availability — e.g., steady-state type training sessions performed at intensities below the lactate threshold. Clearly, more studies are required to investigate the optimal practical approach for which to integrate periods of train-low in an elite athlete's training program.

11.6 CONCLUSIONS

Despite over 100 years of research, CHO metabolism continues to intrigue muscle biologists and exercise scientists. From early recognition as a simple fuel store, it is now apparent that the glycogen granule regulates many cell-signaling processes related to both health and human performance. Nonetheless, it is clear that many of the original questions posed in our field are still relevant today though the array of biochemical tools now at our disposal ensure we are better equipped to answer those questions with greater precision. For example, the storage of the glycogen granule in specific intracellular pools remains a highly active research area. As a related point, the magnitude of exercise-induced utilization of specific storage pools remains to be documented using "real-world" exercise protocols that are relevant to both training and competition scenarios. While the specific regulatory control points of CHO metabolism are now well documented, the precise molecular mechanisms underpinning the regulation of CHO transport, storage, and utilization are not yet fully known. Finally, the identification of a potential muscle glycogen threshold has opened a new field of study that is likely to dominate the applied nature of sport nutrition research in the coming decade. From the early studies from the pioneers in the field (e.g., Krogh, Lindhard, Bergstrom, Saltin), it is clear that our field remains as exciting as ever.

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