

The Study of Cell Signaling Processes in the Process of Body Metabolism

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ABSTRACT

Aim: This research was done with the aim of studying cell signaling processes in the process of body metabolism in 2024.

Methodology: The research method of the present study is descriptive-survey in nature, and applied in terms of research purpose and cross-sectional in terms of time. The research design used in this research is the pre-test-post-test design. The statistical population of this research consists of 3 men and 3 women who are engaged in cycling professionally. In this research, to reach a representative sample, a non-random sampling method was used. In this research, two library and field methods were used to collect data. Sports exercises have been used in the field section.

Results: The findings of this research show that the metabolic process regulates many cells signaling processes that are related to human health and performance. Now that the specific regulatory checkpoints for CHO metabolism are well documented, the precision of the molecular mechanisms regulating CHO transport, storage, and utilization that have not yet been fully identified can be increased. In the process of metabolism, consumed nutrients are analyzed and converted into smaller molecules.

Conclusion: These molecules ultimately contribute to cellular structures such as proteins and nucleic acids. Also, this stored energy is released and transferred to all body activities, including movements and chemical compounds, in signaling processes.

Keywords: Process, Signaling, Cell, Metabolism, Body

INTRODUCTION

Cell signaling is a part of the complex system of communication and exchange of messages in cells, which coordinates and organizes cellular activities. The ability of cells to perceive and recognize their sub-environment, as well as to give the right answer and appropriate reaction to it, is the foundation of growth, tissue regeneration, immunity, and the coexistence of healthy tissues. Various elements have the ability to stimulate cells, including: chemicals and mechanical shock. For example, in the mammalian body, hormones (chemical substances) are able to activate self-related signaling that can ultimately regulate the behavior of stimulated cells. Also, cells must be able to respond to external messages in order to respond to environmental signals, grow and differentiate, produce and secrete proteins and hormones, regulate gene expression, etc. Mistakes in the

processing of cellular data lead to diseases such as cancer and diabetes (35).

The messenger molecule is produced from the outside environment or another messenger cell and is recognized by the receptor of the target cell. The receptor of the target cell receives the extracellular message and converts it into an intracellular message that affects the behavior of the cell. Each cell is exposed to many messenger molecules but responds selectively to these messages. Now this choice depends on the type of receptors that exist on the cell surface. When a messenger molecule binds to a target cell receptor, the intracellular transporters as well as its targets are different in different cells; Therefore, each messenger produces different effects in different cells. On the other hand, each cell has a large number of receptors and therefore responds to several extracellular messengers at the same time;

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Therefore, the combination of different messengers produces different responses. because the intracellular compounds that transmit different messages interact with each other; Therefore, the presence of a messenger can influence the responses of other messengers. Likewise, the speed of the cell's response to different messages is different. If the message affects the activity of proteins and molecules that are already inside the target cell, a rapid response is induced. For example, changes in cell movement, cell secretion, or skeletal muscle contraction. Now, if the answer requires changes in gene expression and new protein production, the answer needs more time. such as increased growth and cell division (1). Metabolism is also a set of chemical changes maintained in the life of the cells of living organisms, which are necessary for biological processes such as cell growth, cell differentiation and homeostasis. The metabolic process is regulated by transcriptional mechanisms that ultimately lead to the production of metabolites. The comprehensive study of metabolites, referred to as metabolomics, increases our understanding of cellular activities and changes in metabolic profiles. Metabolites play an important role in biological systems and can be considered an attractive option for detecting disease phenotypes. Metabolomics with the comprehensive identification of metabolites is considered an excellent tool for diagnosing diseases, identifying new therapeutic targets and creating therapeutic approaches. Metabolites, including amino acids, sugars, nucleotides, lipids, organic acids, perform various functions in a biological system. These metabolites play a role in energy production, synthesis of macromolecules and cell signaling. Currently, how many primary metabolites exist in biological systems is controversial. However, studies show that yeast contains several hundreds of metabolites, humans contain thousands of metabolites, and plants contain hundreds of thousands of metabolites (36).

On the other hand, metabolism or cellular metabolism is defined as biochemical modification of chemical compounds in living organisms and cells. Cellular metabolism is the sum of many individual processes by which living cells process nutrient molecules and maintain their vital functions. A wide variety of metabolic pathways are organized under two headings, anabolism and catabolism. Because anabolic and catabolic processes occur simultaneously in the cell, there are many signals that turn on anabolic processes while turning off catabolic processes. Most of the known signals are hormones and molecules involved in the metabolism process itself. In the past, endocrinology researchers have classified many hormones into two anabolic or catabolic groups. Now, according to the materials mentioned in the statement of the research problem, the researcher is looking for an answer to this basic question: Is there a meaningful relationship between cell signaling processes and the body's metabolic process?

Research method/statistical population and data collection tools

The research method of the present study is descriptive-survey in nature, and applied in terms of research purpose and cross-sectional in terms of time. The research design used in this research is the pre-test-post-test design. The statistical population of this research consists of 3 men and 3 women who are engaged in cycling professionally. In this research, to reach a representative sample, a non-random sampling method was used. In this research, two library and field methods were used to collect data. Sports exercises have been used in the field section.

Research literature

Carbohydrate storage

CHO is mainly stored as glycogen in the liver (approximately 100 g) and muscle (approximately 400 g), and 5 g is also circulated as glucose in the bloodstream. In skeletal muscle, glycogen is typically expressed as mmol · kg⁻¹ of dry muscle weight (d.w) where concentrations in whole muscle homogenate can vary from 50 to 800 mmol · kg⁻¹ depending on training, fatigue, and dietary CHO intake (see Fig. 1). The glycogen granule itself is essentially a tiered structure of glucose units (i.e., polymers) that is formed in a branchlike structure via 1:4 and 1:6 glycosidic bonds. Glycogen granules are formed on the protein glycogen in and can be as large as 42 nm in diameter as well as having potentially 12 tiers. At the maximum size, the granule can consist of 55,000 units of glucosyl. Nevertheless, most glycogen granules have been reported in the human skeletal muscle to a diameter of 25 nm and approximately 8 layers. Although muscle glycogen is traditionally measured through acid hydrolysis throughout the muscle, it is clear, of course, that glycogen is expressed and used in patterns of the specific type of fiber, as well as in specific intracellular locations in the muscle cells themselves. Using histochemical techniques, it has usually been reported that the resting glycogen content apparently does not differ between Type I and Type II fibers. Nonetheless, using biochemical quantification (a more quantitative measure) it has been reported that type II fibers may contain 50-100 mmol. (kg d.w.)⁻¹ more glycogen than type I fibers, 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. In activities involving high-intensity intermittent exercise (e.g., a soccer match), considerable glycogen depletion is observed in both muscle fiber types thus reflecting recruitment patterns to support both moderate and high-intensity running speeds (21). The use of transmission

electron microscopy (TEM) has also shown that glycogen is present in three distinct subcellular pools within myofibrils (intermyofibrillar glycogen, 5–15% of the total glycogen pool), between myofibrils (intermyofibrillar glycogen, 75% of The entire glycogen pool) is stored as well as in the sub-sarcolemma region (sub-sarcolemma). Glycogen, 5-15% of the total glycogen). In endurance-trained athletes, both intermyofibrillar and submicroscopic glycogen stores appear to be greater in type I fibers compared to type II fibers, whereas intermyofibrillar glycogen storage is greater in type II fibers. In relation to acute exercise itself, it is also apparent that intermyofibrillar glycogen stores show a preferential depletion and that failure to restore this specific pool in the immediate hours after exercise are associated with impaired Ca²⁺ release from the sarcoplasmic reticulum (SR) (21). 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.

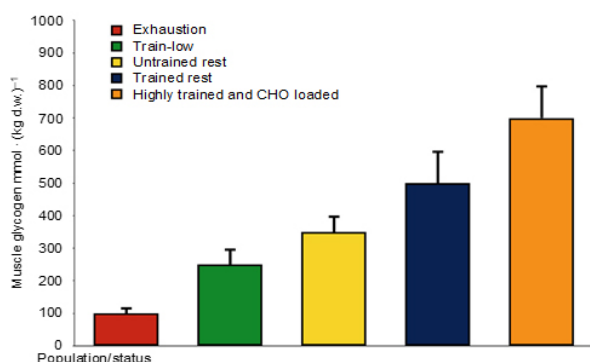


Figure 1: Variations in muscle glycogen storage according to fatigue status, training status, and dietary CHO intake. Data are compiled from studies including (21).

Regulation of carbohydrate metabolism

A review of the key steps in the regulation of CHO metabolism is presented in Figure 2. There are a number of potential locations for control that can regulate the interaction of CHO and lipid metabolism during endurance exercises. These include the availability of intramuscular and extramuscular substrates (controlled by the diet and function of key hormones such as catecholamines and insulin), the abundance of transport proteins that play a role in the transport of substrates through plasma membranes and mitochondria, and, of course, the activity of key regulatory enzymes involved in metabolic pathways. The activity of regulatory enzymes can be modified through covalent modification (for example, phosphorylation and largely hormonal-controlled dephosphorylation) and/or allosteric regulation via important signaling molecules that are produced in the muscle as a result of contraction,

for example, ADP, AMP, IMP, Pi, Ca²⁺, 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 in the long term by increasing muscle cell content of actual enzyme protein (ie, more enzyme actually present), as occurs in endurance training. It is clear that muscle cells have a highly coordinated and regulatory network of signaling and feedback pathways that act to ensure that ATP demand matches 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.

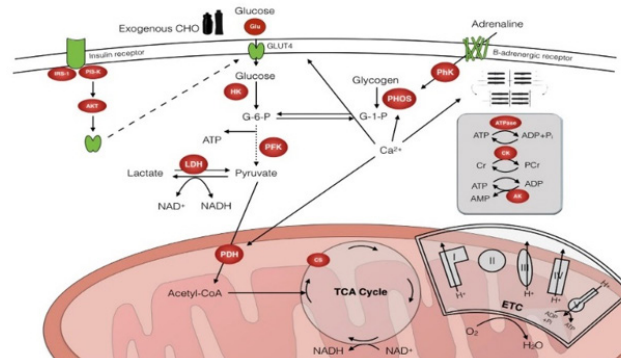


Figure 2: Overview of CHO metabolism and main control points. Taken from Fig. 1, (10)

ADP, adenosine diphosphate; AK, adenylate kinase; Akt, protein kinase B; AMP, adenosine monophosphate; ATP, adenosine triphosphate; Ca²⁺, 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; H₂O, water; IRS-1, insulin receptor substrate 1; HK, hexokinase; LDH, lactate dehydrogenase; O₂, 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.

Effects of Exercise Intensity and Duration

As exercise intensity progress from moderate (i.e., 65% V_{O2}max) to high-intensity (85% V_{O2}max), 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. 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. ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; Ca²⁺, calcium; cAMP, cyclic adenosine monophosphate; GS, G protein; GDP, guanosine diphosphate; GTP, guanosine triphosphate; PKA, protein kinase A; Pi, inorganic phosphate (Fig 3 and 4).

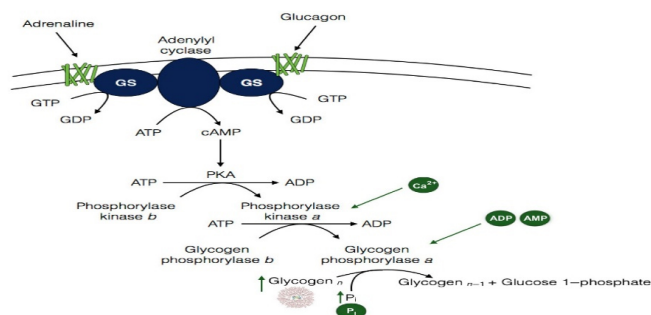


Figure 3: Regulation of glycogen phosphorylase activity, Positive allosteric effectors are shown in green.

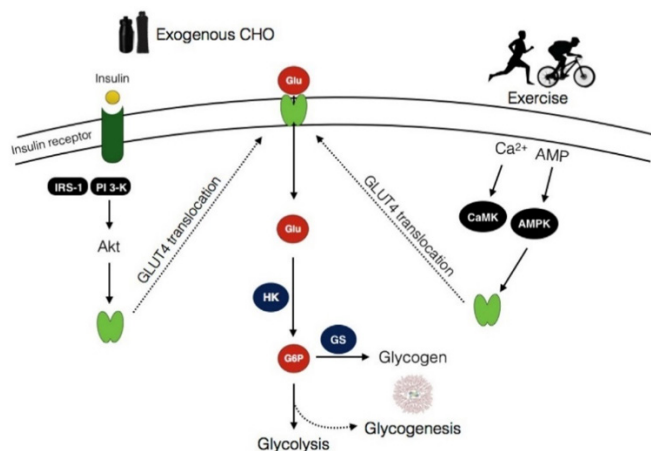


Figure 4: Regulation of muscle glucose uptake.

AMP, adenosine monophosphate; AMPK, 5' adenosine monophosphate-activated protein kinase; Akt, protein kinase B; Ca²⁺, 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 (Fig 5).

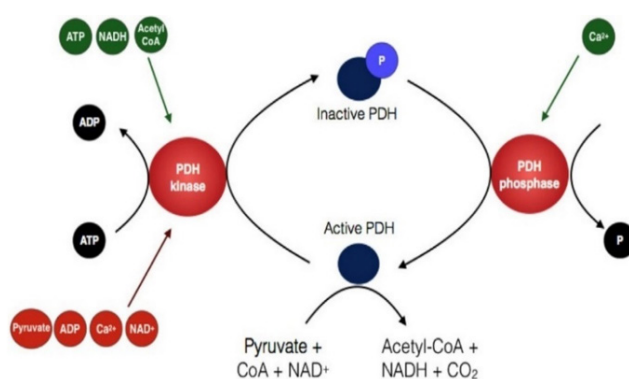


Figure 5: Regulation of PDH activity.

ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ca21, 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 Ca21 levels would be increased with high-intensity exercise (given the need for more rapid cross-bridge cycling) and that Ca21 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.

While this conversion mechanism is mediated by the onset of contraction, post-conversion mechanisms appear to operate at high intensity over longer periods of exercise, given that glycogenolysis still occurs despite the decrease in conversion. In this regard, vital signals related to the energy state of the cell play a more prominent role. In fact, as the intensity of exercise progresses from moderate-intensity exercise to high-intensity exercise, the hydrolysis rate of ATP increases so much that there is a greater accumulation of ADP, AMP, and Pi. In this way, increasing the accumulation of Pi as a result of increased hydrolysis of ATP can increase glycogenolysis as it increases the substrate required for the reaction. In addition, further accumulation of free ADP and AMP can subsequently regulate the activity of phosphorylase a through allosteric regulation. Finally, although it is well documented that phosphorylase is under hormonal control of adrenaline, injecting adrenaline into levels beyond endogenous production during high glycogenolysis is likely due to adequate activation beforehand 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 thus substrate delivery) in addition to increased muscle fiber uptake. Although glucose uptake is also regulated by Glut4 content, it is likely that Glut4 does not play a role in this situation because the transfer of Glut4 to the plasma membrane does not increase with exercise intensity. When glucose is transported to the cytosol, it is phosphorylated to glucose-6-phosphate under the control of hexokinase. Evidence suggests that hexokinase activity is also not limiting, given that patients with type 2 diabetes (who have maximally reduced hexokinase activity) exhibit normal patterns of exercise-induced glucose uptake due to normal perfusion and Glut4 transport. 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. Once glucose enters the glycolytic pathway, the rate-limiting enzyme to glycolysis is considered as phospho- fructokinase (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 (36).

In contrast to exercise intensity, prolonged steady-state exercise lasting several hours is characterized by a shift toward increased lipid oxidation and reduced CHO oxidoregulators of fuel utilization patterns during exercise and this concept is discussed in the next section.

Effects of Substrate Availability

Changing the availability of substrates through dietary manipulation (such as loading diets, pre-workout meals or providing increased bed availability during workout) has been consistently shown to alter metabolic regulation during endurance workout through various control points. Increased concentrations of muscle glycogen increase glycogenolysis during exercise. With increased phosphorylase activity given that glycogen is a substrate for phosphorylase. Increased glycogenolysis with increased glycogen reserves does not seem to affect the absorption of muscle glucose. In addition to glycogenolysis muscle glycogen also appears to be a strong regulator of PDH activity (And thus the oxidation of CHO) during exercise. In fact, starting exercise by reducing muscle glycogen reduces the increase in PDH activity caused by exercise, and vice versa, probably due to a decrease in glycolytic flux, as well as an increase in the resting content of PDK4 (kinase responsible for disabling PDH) when glycogen concentrations are low. The PDH setting seems to be very sensitive to the feeding state 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. 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 (24).

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 (26).

In addition, more recent data demonstrate an up-regulation of PDH kinase activity during exercise which would therefore directly inhibit PDH activity. Taken together, these data are consistent with the many observations that increasing or decreasing substrate availability is one of the most potent commenced with high glycogen (591 mmol (kg d.w.)²¹) as opposed to exercise at 70% $\dot{V}_{O_2}$ max commenced with low glycogen concentration (223 mmol (kg d.w.)²¹) 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. 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 (17).

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

(34). 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. Consequently, it is common practice for athletes to adopt such dietary approaches to competition. However, it is now well documented that eating choices before and during exercise is one of the most powerful ways to alter the pattern of CHO utilization during exercise through a number of checkpoints. One of the main responses to CHO feeding is to decrease plasma ffa availability and lipid oxidation while simultaneously increasing the rate of CHO oxidation. Decreased availability of plasma FFA is due to decreased lipolysis, which is regulated by increased circulating insulin concentrations induced by CHO feeding. The anti-lipolytic effect of insulin is mediated through its ability to activate the enzyme phosphodiesterase, which degrades cAMP, thereby reducing the activation of protein kinase A and ultimately hormone-sensitive lipase (HSL).

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 · kg⁻¹ of glucose (to induce a high insulin response), 0.8 g · 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 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) compared with the glucose only trial (3.1 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) but were still not restored to levels occurring during fasted exercise (6.1 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Taken together, while these data suggest that only small elevations in insulin can attenuate lipolysis (i.e., 10-30 $\mu\text{U}/\text{mL}$), 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 were 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 (5).

In an effort to ascertain the source of limitation to lipid oxidation within the muscle following CHO feeding, 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 · 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, thus highlighting an additional point of control (8).

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.

For a certain exercise intensity, the use of glycogen is reduced by exercise exercises, an effect that is locally limited to the actual muscles practiced. The decrease in glycogenolysis observed after training was not due to any changes in phosphorylation conversion, but due to allosteric mechanisms. In fact, exercise in the practiced state is associated with a decrease in ADP, AMP and Pi content, thereby providing a mechanism that leads to a decrease in phosphorylase activity. A decrease in pyruvate and lactate production was also observed during exercise performed in the practiced mode,

as well as a decrease in PDH activity. As a result of decreased CHO flux, it is therefore likely that poor pyruvate production (in addition to decreased ADP accumulation) may have reduced PDH activity (14). In addition to exercise-induced reductions in muscle glycogenolysis, several researchers have observed that exercise reduces exercise-induced liver glycogenolysis, as evidenced by the rate at which glucose appears in the circulation. There is evidence (although this is not consistent in the literature) that endurance exercises also increase the gluconeogenesis of skeletal muscles after training. In accordance with the decrease in the production rate of glucose, the absorption of muscle glucose decreases when exercise is performed with the same absolute workload and after a period of endurance training.

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. 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. Rather, it is possible that the actual rate of muscle glucose uptake acts as a feedback signal to regulate glucose output from the liver (7).

Carbohydrate performance and exercise

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 (23).

Muscle Glycogen and Carbohydrate Loading

The basics of CHO loading were developed in the late 1960s, where it was found that a period of boring exercise followed by a few days of excessive consumption of CHO in the diet creates an incredibly compensatory effect so that the storage of glycogen increases. It is now generally accepted that trained athletes can increase glycogen storage in both Type I and II fibers within 24-48 hours after increased CHO intake. In relation to practical application it is also accepted that high-glycemic foods have an advantage over low-glycemic foods in augmenting glycogen storage and that dietary intake of 8-12 g · kg⁻¹ per day are required (32). 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. 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 (18).

Indeed, in reviewing the literature 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 intermyofibrillar storage pool) can directly modulate contractile function. Indeed, a series of studies from have collectively shown a preferential utilization of this storage pool during exercise in a manner that also correlates with impaired Ca²⁺ 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 (22).

Preexercise Carbohydrate Availability

Preexercise feeding (i.e., 3-4 h before competition) is not only advantageous as it can lead to further elevations in muscle glycogen content 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 observed that time-trial performance after 90 min of steady-state exercise at 70% V_{O₂max} 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. As such, current CHO guidelines for preexercise feeding advise an intake of 1-4 g · kg⁻¹ body mass 3-4 h prior to exercise (32).

Carbohydrate Feeding During exercise

In addition to endogenous pre-exercise muscle glycogen stores, it is widely accepted that exogenous chow nutrition during exercise also improves the physical, cognitive, and technical elements of performance. While it was generally accepted that the rate of external CHO oxidation is limited to about 1 g · min⁻¹ due to saturation of intestinal glucose transporters, it is now known that the rate of external CHO

oxidation can be increased by adding sucrose or fructose to the CHO mixture. increase to $1.8 \text{ g} \cdot \text{min}^{-1}$. When taken together, it is thought that CHO feeding during exercise may enhance exercise performance through multiple mechanisms including muscle glycogen sparing, liver glycogen sparing, and maintenance of plasma glucose and CHO oxidation. 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 (29).

It is noteworthy that exogenous CHO feeding during exercise also improves performance 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. Such data suggest that CHO feeding may also improve exercise performance via nonmetabolic effects but through direct effects on the central nervous system. 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, an effect that is independent of sweetness and that is especially apparent in the absence of a preexercise CHO meal and low preexercise muscle glycogen (15).

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. 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 $30\text{-}90 \text{ g} \cdot \text{h}^{-1}$ depending on exercise duration. Nevertheless, although there is little difference in exogenous CHO oxidation rates (albeit in fluid matched conditions) between the aforementioned sources, 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 as well as the presence of fiber, fat, and protein in energy bars. 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 $30\text{-}60 \text{ g} \cdot \text{h}^{-1}$ of CHO (glucose polymers) is consumed during events lasting, 60-90 min whereas in events.2-3 h, $60\text{-}90 \text{ g} \cdot \text{h}^{-1}$ (glucose/fructose blends) is the recommended rate (32).

Carbohydrates and training adaptation

A review of the molecular regulation of educational 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 ($\dot{V}\text{O}_2\text{max}$) 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 (25).

This increase in mitochondrial mass eventually allows endurance athletes to exercise at higher intensity for longer periods of time. An overview of the proposed mechanisms that make up the adaptive skeletal muscle responses to exercise is shown in Figure 5. In response to any individual sports competition, acute transcription changes in the muscle occur during recovery hours, and it is the accumulation of these acute responses over time that subsequently changes the muscle to an oxidative phenotype through the expression of new proteins. When muscle contraction begins, a number of metabolic disorders occur in muscle cells (e.g., an increase in amp/ATP ratio, Ca^{2+} flux, lactate, hypoxia, and energy availability), which in total triggers 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. The most well-studied regulator of mitochondrial biogenesis is the transcriptional coactivator peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α). The importance of PGC-1 α as a mediator of mitochondrial biogenesis is evident from rodent studies demonstrating that overexpression increases oxidative enzyme activity and improves exercise capacity. In humans, elevated PGC-

1 α mRNA levels are observed following endurance exercise with the highest abundance typically present in the first 2-4 h of recovery (2).

Both AMPK and p38MAPK can directly phosphorylate PGC-1 α 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 (ERR α), peroxisome proliferator-activated receptor (PPAR δ), 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-1 α 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. In addition to PGC-1 α , the tumor suppressor protein p53 has now emerged as a potential regulator of mitochondrial biogenesis. Indeed, acute exercise induces the posttranslational modification of p53, and similarly to PGC-1 α , this protein also translocates to the nucleus and the mitochondria to interact with Tfam (28).

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 lipid metabolism and can improve both exercise capacity and performance. 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 (19).

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, 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. 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.

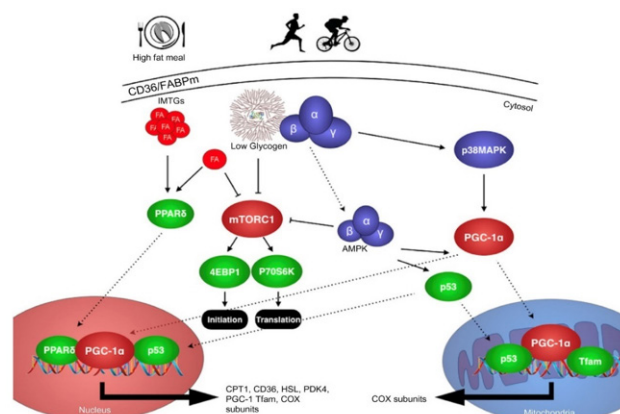


Figure 6: Overview of key molecular signaling pathways regulating endurance training adaptations.

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, explored this idea with participants completing 75-min of cycling 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 at 75% V_{O2}max followed by the consumption of a diet group. Despite significant increases in CS and β -HAD 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. When taken together, responses from these studies suggest that reducing CHO availability in the recovery period also modulates the muscle adaptive process.

Twice-per-day Training Models

On the basis of the molecular evidence derived from acute studies, 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 (16).

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 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. (11) also reported greater increases in intramuscular lipid oxidation and the expression of CD36 and β -HAD following training low compared with training high.

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. (3), 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-1 α 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, 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. 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. (19) 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, with data suggesting that AMPK is more active when glycogen is depleted. Findings Lane et al (16), indeed demonstrated that when preexercise muscle glycogen levels are reduced, AMPK α 2 activity and ACCSer221

phosphorylation are significantly elevated following steady-state cycling compared to when muscle glycogen is high. In a subsequent study, also observed a significantly greater nuclear abundance of p38MAPK both pre- and postexercise when muscle glycogen levels were low compared too high. In another twice-per-day train-low study, 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-1 α activation.

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 (4). Further studies have also observed increases in resting intramuscular triglyceride stores, HSL, AMPK- α 2 activity (34), and increases in the protein content of CD36 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 (9).

Nonetheless, it is noteworthy that high-fat feeding may actually impair glycogen utilization during exercise. Indeed, 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. 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. (6) 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. 5) (20). In this regard, data collected from lipid and heparin infusion suggests that high circulating FFA availability actually impairs muscle protein synthesis 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- 1 α , p53, Tfam, CS, and ERR α were not different between trials, suggesting no additional benefit high-fat feeding during periods of train-low (30).

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 $\cdot$ (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 $\cdot$ (kg d.w.)⁻¹) glycogen (see Fig. 6). As such, it would appear important that exercise is commenced with muscle glycogen concentrations below 350 mmol $\cdot$ (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) (13). For instance, Impey et al. (12) demonstrated that exhaustive exercise induces significant activation of AMPK and expression of transcription factors (p53, Tfam) and coactivators (PGC-1 α), even when commenced with high glycogen levels (600 mmol $\cdot$ (kg d.w.)⁻¹). This is likely due to the fact that subjects surpassed a critical level of glycogen (B350 mmol $\cdot$ (kg d.w.)⁻¹) during exercise and reached exhaustion at very low levels (B100 mmol $\cdot$ (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 (B300 mmol (kg d.w.)⁻¹) glycogen induces a significant activation of AMPK in significantly less time (B60 min) than when training is commenced with high glycogen.

ACC, acetyl-CoA carboxylase; AMPK, 5' adenosine monophosphate-activated protein kinase; Ca²⁺, 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-1 α , peroxisome proliferator-activated receptor gamma coactivator 1-alpha; Tfam, transcription factor A (22).

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. 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. 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. (13) demonstrates that p70S6K activity is suppressed when glycogen concentrations reach very low levels (B100 mmol · (kg d.w.)⁻¹) despite feeding leucine enriched whey protein. However, repletion of muscle glycogen to B250 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. 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

Ca²⁺ transients in a subsequent fatigue test. These findings have been subsequently confirmed in human skeletal muscle, where an impairment in SR Ca²⁺ release rate and subsequent power output are apparent under conditions of low muscle glycogen (7). 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 Ca²⁺ 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.

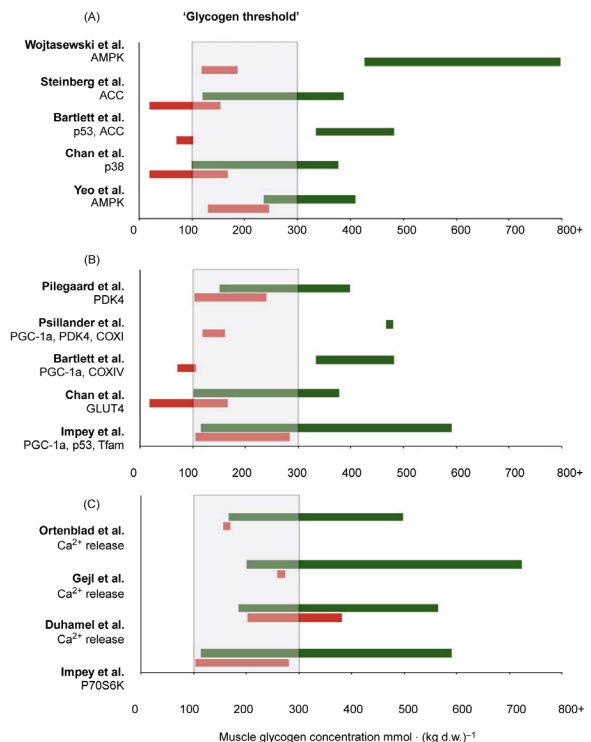


Figure 7: Summary of studies demonstrating differential metabolic responses of skeletal muscle in response to exercise commenced in conditions of high or low muscle glycogen availability. Taken from Fig.2, (13).

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 (11) 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. Restriction of CHO availability has also been shown to increase muscle protein breakdown (27), 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 (Taylor et al., 2013). 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.

CONCLUSION

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, it is clear that our field remains as exciting as ever.

Also, The findings of this research show that the metabolic process regulates many cells signaling processes that are related to human health and performance. Now that the specific regulatory checkpoints for CHO metabolism are well documented, the precision of the molecular mechanisms regulating CHO transport, storage, and utilization that have not yet been fully identified can be increased. In the process of metabolism, consumed nutrients are analyzed and converted into smaller molecules. These molecules ultimately contribute to cellular structures such as proteins and nucleic acids. Also, this stored energy is released and transferred to all body activities, including movements and chemical compounds, in signaling processes.

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Conflict of Interest

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