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REVIEWS | *Intracellular Signal for Skeletal Muscle Adaptation*

More than a store: regulatory roles for glycogen in skeletal muscle adaptation to exercise

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Philp A, Hargreaves M, Baar K. More than a store: regulatory roles for glycogen in skeletal muscle adaptation to exercise. *Am J Physiol Endocrinol Metab* 302: E1343–E1351, 2012. First published March 6, 2012; doi:10.1152/ajpendo.00004.2012.—The glycogen content of muscle determines not only our capacity for exercise but also the signaling events that occur in response to exercise. The result of the shift in signaling is that frequent training in a low-glycogen state results in improved fat oxidation during steady-state submaximal exercise. This review will discuss how the amount or localization of glycogen particles can directly or indirectly result in this differential response to training. The key direct effect discussed is carbohydrate binding, whereas the indirect effects include the metabolic shift toward fat oxidation, the increase in catecholamines, and osmotic stress. Although our understanding of the role of glycogen in response to training has expanded exponentially over the past 5 years, there are still many questions remaining as to how stored carbohydrate affects the muscular adaptation to exercise.

diabetes; metabolism; peroxisome proliferator-activated receptor; adenosine 5′-monophosphate-activated protein kinase; peroxisome proliferator-activated receptor-γ coactivator-1α

THE STUDY OF GLYCOGEN has a long and storied history. From its discovery as the principal storage form of carbohydrate in 1858 (9) to the first signaling pathway regulating its mass [leading to the Nobel prize for medicine in 1947 (20)] to its implicit relationship with muscle contraction and fatigue (4), this sugar polymer has intrigued biologists for more than a century and a half. From this intense research, it has become clear that glycogen is not only a vital form of energy for periods of metabolic stress but also an important molecular signal that regulates enzyme activity, protein function, gene expression, and adaptation to exercise.

The interest in glycogen from an exercise perspective began with the seminal studies of Bergström and colleagues (4–8) and Hermansen et al. (42). They demonstrated that glycogen content was decreased following muscle contraction (5), that glycogen depletion was a key factor in skeletal muscle fatigue (4), that glycogen resynthesis was enhanced following glycogen depletion (supercompensation) (6), that glycogen was the primary carbohydrate used at high workloads (42), and that the glycogen content of the working muscle is a major determinant of the capacity to sustain long-duration exercise (7). Importantly, Bergström et al. (4) also demonstrated that diet and exercise intensity could greatly vary the glycogen content in skeletal muscle, which then affected exercise capacity. Finally, they reported that ingestion of a high-carbohydrate diet following exercise increased the recovery of muscle glycogen stores compared with a fat or protein diet, suggesting that dietary glucose could increase muscle glycogen (8, 47).

In the last decade, the structure (82), subcellular localization (72), regulation (85), and components of the glycogen complex (34) have been described in detail. Recent identification of glycogen-binding domains, regions within proteins that interact specifically with glycogen, and defined promoter regions in genes that are sensitive to glycogen levels or glucose derived from glycogen, termed carbohydrate response elements, has added additional complexity to glycogen's regulatory roles (12). There is emerging evidence that these molecular regulatory mechanisms either directly or indirectly link glycogen content to skeletal muscle adaptation in response to acute and chronic exercise. Accordingly, within this review we will highlight recent advances supporting the effect of glycogen on transcriptional and posttranslational processes in skeletal muscle while discussing the direct and indirect mechanisms that underlie the effects of glycogen on skeletal muscle adaptation to exercise. Given space constraints, we will not discuss the role of glycogen in the regulation of insulin sensitivity, which has been expertly reviewed elsewhere (50).

Regulation, Design, and Localization of the Glycogen Granule

The glycogen molecule is an elegant example of an optimized, highly efficient cellular energy storage system. The branched structure allows the dense compartmentalization of free glucose,

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providing muscle or liver with a readily accessible form of energy (66). By accumulating the glucose into a polymer, the glycogen granule allows a large amount of glucose to be stored without cellular osmolarity being significantly affected (66). Approximately 400 mM of glucose can be stored in 0.01 μM liver glycogen (41). In addition, glycogen branching means that each granule contains a large surface area, allowing for rapid degradation. Each glycogen molecule can contain ~55,000 glucose residues in an area of 8,000 nm^3 (33). Indeed, this branching design is critical, since a particle that is too dense would not degrade because phosphorylase, the protein that initiates degradation, would not be able to access the proper branches. Thus, regulation of the branch length, number, and density appears to be a highly regulated and conserved process (66).

Beyond the amount and shape of the glycogen particle, its location within a muscle also appears to be important. The majority of exercise studies have measured total glycogen content in muscle using acid-based digestion of glycogen, followed by enzymatic determination of free glucose. Although this approach has been rewarding in studying glycogen-mediated regulation of whole muscle metabolism, it does not allow for examination of glycogen localization or compartmentalization. Understanding glycogen localization has been achieved through the use of transmission electron microscopy (TEM) on muscle sections. This approach has led to the appreciation that glycogen is located in specific cellular regions (78). Within skeletal muscle, glycogen is distributed principally in *1*) the intermyofibrillar space, *2*) the intramyofibrillar space, and *3*) the subsarcolemmal compartment (63). TEM also allows the quantification of glycogen particle size, number, and density (63). These elegant studies have demonstrated that glycogen localization is highly ordered within muscle and forms an energy complex, associating with intramuscular triglyceride (IMTG) deposits and mitochondria (84, 93, 95). Physiologically, this organization places muscular energy stores in close proximity to their site of utilization. This localization has also led to speculation that glycogen content (by physical interaction) may influence mitochondrial function and IMTG content, although presently this interaction has not been tested experimentally.

Beyond the formation of an energy complex with IMTG, the subcellular localization of glycogen potentially provides a substrate for specific cellular functions. For example, the intramyofibrillar pool of glycogen is positioned to provide carbohydrate for cross-bridge cycling, is preferentially depleted during high-intensity exercise (71), and correlates with muscle fatigue (72). In contrast, depletion of the intermyofibrillar glycogen fraction (located close to the sarcoplasmic reticulum, T-tubules, and mitochondria) correlates with the half-relaxation time in fibers (72), suggesting that it is important in driving the repolarization of the T-tubules through the provision of energy for the Na-K-ATPase and the sarco(endo)plasmic reticulum calcium ATPase. Presently, the role of the subsarcolemmal glycogen fraction in fatigue is less clear; however, its localization and sensitivity to exercise and nutrition suggest that it could play a role in cellular signaling.

Glycogen-Associated Proteins: Defining the "Glycogen Proteome"

A number of proteins have been reported to directly associate and localize with glycogen (Table 1). The interaction and regulation of these proteins is complex. For simplicity, we will categorize these as proteins that are either *1*) involved directly in the generation or regulation of the glycogen granule or *2*) metabolic proteins that appear to be regulated by glycogen content. Discussing this first subset of proteins is beyond the scope of the present review; instead, we direct the reader to recent expert viewpoints on the subject (34, 78).

Table 1. *Select proteins from the glycogen proteome in liver*

Protein
Glycogen metabolism
Glycogenin
Glycogen-branching enzyme
Glycogen-debranching enzyme
Glycogen synthase
Glycogen phosphorylase
Protein phosphatase-1α catalytic subunit α-amylase
Other metabolic proteins
ATP synthase subunit-α
ATP synthase subunit-β
ADP/ATP translocase 1
Malate dehydrogenase
Glyceraldehyde-3-phosphate dehydrogenase
Fructose-bisphosphate aldolase B
AMP-activated protein kinase
Protein synthesis/degradation
Cathepsin B
Elongation factor-α1
40S ribosomal protein S18
60-kDa heat shock protein
Other functions
Glucose-regulated protein 78 (BiP)
Glutathione *S*-transferase
Catalase

The glycogen proteome, those proteins that directly interact with glycogen, was recently determined from rat and mouse liver (88). Those authors identified ~70 proteins that associate with hepatic glycogen. As anticipated, proteins known to be involved in glycogen breakdown and synthesis (glycogen phosphorylase, glycogen synthase, glycogen-branching enzyme) were identified in the screen. The screen also identified a number of novel glycogen-interacting proteins (Table 1). When categorized by physiological function, these proteins had a diverse array of functions ranging from metabolism to redox balance, RNA processing, and protein synthesis. Of the metabolism subset, proteins involved in fat metabolism (long-chain fatty acid-CoA ligase 1) and oxidative phosphorylation (ATP-synthase α/β, NADH-cytochrome b5 reductase 3) were identified, suggesting that glycogen may directly associate and potentially regulate the process of substrate utilization. It will be interesting to see whether similar proteins associate with skeletal muscle glycogen preparations and to determine whether glycogen regulates the activity of these proteins.

Within the subset of metabolic proteins that are known to interact with glycogen, the mammalian AMP-activated protein kinase (AMPK) has received most attention with regard to glycogen content. AMPK is an αβγ heterotrimer with multiple genes encoding each of the subunits (94). AMPK activity appears to be regulated by three fundamental processes: *1*) binding of AMP, ADP, or ATP to the γ-subunit (94), *2*) phosphorylation of the catalytic α-subunit by a number of upstream kinases (90), and *3*) a glycogen-binding domain (GBD) located on the β-subunit (45, 76, 77). Whereas regula-

tion of the α- and γ-subunits has been examined extensively, less is known about the physiological relevance of the GBD in the β-subunit (65).

The functionality of the GBD has been explored recently by McBride et al. (65), who demonstrated that incubation of AMPK with isomaltose, a carbohydrate that mimics the branch points of glycogen, inhibited the AMPK activity by 33%. This observation led these authors to suggest that AMPK can sense the branching structure of glycogen, leading to suppression of the kinase. Recently, Koay et al. (58) demonstrated that AMPK can also associate with glycogen via a carbohydrate-binding module (CBM) in the β_2-subunit. Deletion of the AMPKβ2 Thr^{101} motif within the CBM reduced the affinity of AMPK for single α-$_{1-6}$ branched oligosaccharides threefold. Inserting the Thr^{101} motif into the AMPKβ_1 subunit resulted in an increase in glycogen binding, confirming the functionality of the domain (58). However, whether the CBM can regulate AMPK signaling has yet to be determined.

As would be suggested by the effect of glycogen on AMPK activity, both the basal and postexercise activities of AMPKα2 are higher in the glycogen-depleted state (103). The ingestion of sufficient glucose to spare glycogen attenuates AMPK activation ~50% compared with a placebo trial (1). However, when a similar glucose ingestion trial is performed but glycogen sparing does not occur (i.e., cycling exercise where a similar feeding paradigm did not alter glycogen use), AMPKα2 activity is not affected (59), suggesting that the amount of glycogen within the muscle directly modulates AMPK activity. Steinberg et al. (91) demonstrated that exercise in a glycogen-depleted state also leads to nuclear translocation of AMPKα2 and subsequent increases in glucose transporter 4 (GLUT4) mRNA expression. Yeo et al. (104) also found that AMPK Thr^{172} phosphorylation was greater in trained cyclists when high-intensity exercise was performed in a glycogen-depleted state. Taken together, these data suggest that AMPK may play an important role in the metabolic adaptations to low-glycogen exercise. However, it should be noted that the training-induced increase in GLUT4, one of the key metabolic targets of AMPK, is decreased following low-glycogen training, suggesting that AMPK-independent mechanisms are also important in the metabolic adaptation following exercise in a glycogen-depleted state.

Glycogen Content, Substrate Turnover, and the Adaptation to Exercise

In the low-glycogen state, whole body metabolism shifts drastically (11, 40, 100, 102). In humans, glycogen depletion results in increased systemic release of amino acids from muscle protein breakdown, increased fat metabolism (calculated from arterio-venous differences), and reduced pyruvate oxidation (11). Steensberg et al. (89) reported an increase in plasma free fatty acids, epinephrine, and cortisol concentrations between 90 and 120 min of exercise in a glycogen-depleted state. This led these authors to postulate that lower glycogen per se altered whole body substrate metabolism and stimulated the activation of cellular signaling pathways that might be involved in the muscular adaptation to training.

Hansen et al. (39) were the first to test directly the effect of skeletal muscle glycogen content on training adaptations. To achieve this, these authors employed an elegant contralateral leg-kicking model in which one leg trains twice a day, every other day (low glycogen), compared with the contralateral leg that trained once daily (normal glycogen). The benefit of the twice a day model is that the second bout is performed in a low-glycogen state. Utilizing this approach, Hansen et al. (39) demonstrated that 10 wk of training with low muscle glycogen increased endurance (time to exhaustion) and oxidative capacity (citrate synthase activity) and tended to increase 3-hydroxyacyl-CoA dehydrogenase activity compared with training with high-muscle glycogen in all sessions. Two independent groups have extended this approach to a trained athlete model (46, 105). As in the study by Hansen et al. (39), the subjects performed six training bouts per week. However, in these studies one half of the training bouts was long steady-state rides at 70% maximal aerobic capacity ($\dot{V}O_{2max}$), and the other half was high-intensity interval workouts. In the high-glycogen groups, the athletes exercised every day, alternating between steady state and high-intensity training (HIT). In the low-glycogen groups, the subjects trained every other day, performing the HIT in a low-glycogen state 1 h after the steady-state exercise (46, 105). As would be expected, athletes who undertook HIT with ~50% lower muscle glycogen showed significantly lower performance during these sessions (12, 13). However, following the 3-wk training period, 60-min time trial performance improved to the same extent in the low- and high-glycogen groups, indicating that relative to their training intensity the low-glycogen group showed a greater adaptation. More interestingly, during steady-state exercise at 70% $\dot{V}O_{2max}$, the low-glycogen group showed greater lipid oxidation, which from tracer analysis appears to be the result of increased IMTG utilization (46). Together with a shift toward fatty acid oxidation, there was glycogen sparing and a greater increase in succinate dehydrogenase and 3-hydroxyacyl-CoA dehydrogenase enzyme activity (46, 105). Together, these data indicate that, regardless of training state, high-intensity exercise with low muscle glycogen improves the capacity for fatty acid oxidation to a greater degree than training with normal glycogen levels.

The key question that remains unanswered is whether the proadaptive responses induced during these twice a day, every other day training protocols are due to alterations in the cellular environment that ultimately lead to enhanced signaling or simply to having a greater load and increased recovery period on each training day. If we consider the first scenario, an examination of exercise studies that have directly or indirectly manipulated glycogen content has shown that exercise in a low-glycogen environment is associated with *1*) elevated plasma free fatty acids (FFA), *2*) increased sympathetic nervous system activity, *3*) hyperosmotic stress (which results in elevated cellular stress) due to the loss of water associated with glycogen, and *4*) increased myokine production (Fig. 1). The potential effects of these changes to the cellular milieu will be discussed below.

Exercise in a glycogen-depleted state leads to increased whole body and skeletal muscle lipid metabolism. One of the reasons for this shift is the liberation of FFA from adipose and intramuscular storage sites. The increased circulating FFA are important because they serve two roles: *1*) as substrates for β-oxidation in the mitochondria (55) and *2*) as signaling intermediates for transcription factors and nuclear receptors that regulate the proteins involved in the transport and breakdown of lipids (55). Understand-

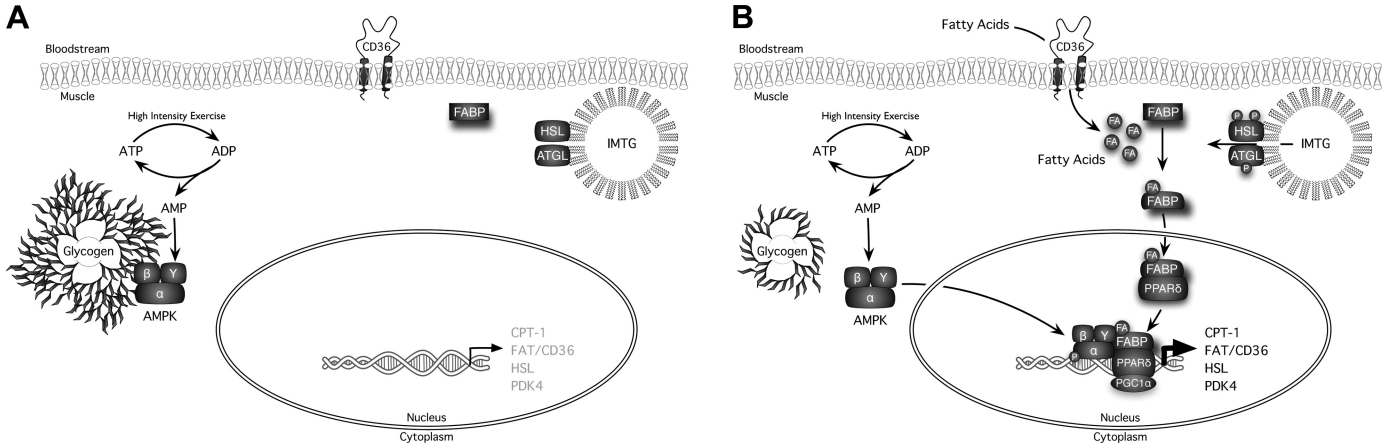

Fig. 1. Graphic representation of some of the signaling events that are differentially activated by exercise in either a high-glycogen (*A*) or low-glycogen state (*B*). In a low-glycogen state, the greater catecholamine response results in the activation of protein kinase A and the phosphorylation and removal of the carbohydrate response element-binding protein and the sterol response element-binding protein from the nucleus. In addition, exercise in a low-glycogen state leads to the phosphorylation and activation of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) and the activation of peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC-1α). The increase in circulating fatty acids (FA) and the breakdown of intramuscular triglycerides bind to and activate the PPARδ, resulting in an increase in the synthesis of mRNA associated with greater fat oxidation such as carnitine palmitoyltransferase (CPT I), FA translocase (FAT/CD36), and HSL. The decrease in glycogen content also results in the release of the AMP-activated protein kinase (AMPK) from the glycogen particle, resulting in greater activity and altered localization. Finally, the osmotic stress associated with a rapid change in glycogen content can activate mitogen-activated protein kinases such as p38, which can phosphorylate and activate PGC-1α. Together, these alterations in muscle signaling result in improved FA utilization that ultimately results in glycogen sparing during steady-state exercise. FABP, FA-binding protein.

ing the molecular cross-talk between the adipocyte and myocyte is an area of intense investigation. Two proteins that appear to be important in the initial breakdown of lipid droplets in both adipose and muscle are adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). The complex regulation of these proteins has been expertly reviewed elsewhere (99); however, the action of these proteins in the hydrolysis of lipid stores is important in the context of this review because these liberated fatty acids can serve as endogenous ligands for the peroxisome proliferator-activated receptor (PPAR) nuclear receptors-α and -β/δ (PPARα/δ) (Fig. 1).

There is limited information regarding the role of PPARδ in skeletal muscle metabolic regulation. PPARδ is the most abundant PPAR in skeletal muscle and has been shown to be enriched in oxidative type I fibers (13). Given the association of PPARδ expression with skeletal muscle oxidative capacity, it is not surprising that both acute (62, 98) and chronic (30, 81) exercise increase PPARδ mRNA expression in both rodent and human skeletal muscle. Therefore, these observations have led to the suggestion that PPARδ activity is under the control of an exercise-derived factor (23). When PPARδ is activated, it controls the transcription of fatty acid oxidation enzymes, including *1*) carnitine palmitoyltransferase I (32), *2*) forkhead box O1 (70), *3*) fatty acid translocase (43), and *4*) hormone-sensitive ligase (87). Fyffe et al. (31) have shown that long-chain FFA (carbon length: C16:0, C16:1, C18:0, and C18:1) can activate PPARδ via its protein ligand-binding domain. However, simply treating C_2C_{12} myotubes with long-chain FFA failed to increase PPARδ expression (44). In contrast, Kleiner et al. (57) demonstrated that in primary mouse myoblasts the PPARδ-synthetic ligand GW-501516 increased fatty acid oxidation in a PPARδ-dependent manner via upregulation of key lipid target genes. Collectively, these data would suggest that the exercise-induced increase in the activity of PPARδ target genes may be facilitated by binding of a long-chain FFA to PPARδ. Given that exercise in a low-glycogen state increases circulating C16 and C18 FFA, determining whether such FFA or species derived from these FFA are the endogenous PPARδ ligand is a key question to be addressed. Furthermore, if PPARδ activity was increased during low-glycogen conditions, it could in part explain aspects of the adaptive increase in lipid oxidation when training is performed in this cellular environment.

Because glycogenolysis is tightly regulated in skeletal muscle, when glycogen stores are low, the body responds to this metabolic stress and initiates response programs designed to maintain energy provision. An example of this is the elevation in circulating catecholamine (epinephrine and norepinephrine) levels observed during low-glycogen exercise (97). Increased catecholamine levels promote an increase in fat metabolism by activating HSL through protein kinase A (PKA). HSL is phosphorylated by PKA on three sites (Ser^{563}, Ser^{659}, and Ser^{660}) (56). Although it is not clear how these sites regulate HSL activity, increased HSL activity drives lipolysis in both adipose tissue and skeletal muscle. The result is the liberation of FFA from both adipose and intramuscular depots (56).

An additional cellular target of catecholamine action is the cAMP response element-binding protein (CREB). Exercise can increase the phosphorylation and activation of CREB in both exercised muscle and muscles that were not recruited during the exercise (101) due to the central effects of elevated sympathetic nervous system activity. One of the targets of CREB is the transcriptional coactivator PPARγ coactivator-1α (PGC-1α). PGC-1α has been suggested as a master regulator of mammalian mitochondrial biogenesis (37) due to its ability to interact with and facilitate transcriptional signaling in response to extrinsic stimuli. Akimoto et al. (2) demonstrated that the CREB site within the PGC-1α promoter is required for the exercise-induced increase in PGC-1α. Miura et al. (67) extended this work to show that blocking β-adrenergic receptors with ICI-118,551 prevented 69% of the exercise-induced increase in PGC-1α. Furthermore, the induction of PGC-1α following exercise was lower in mice lacking β-receptors than in wild-type mice (67). Not only is PGC-1α mRNA increased

by catecholamines, the PGC-1α mRNA that is made in response to catecholamines comes from a different promoter and may have a higher activity (17). Together, these data suggest that catecholamines acting through β-adrenergic receptors may play a significant role in the increase in fatty acid oxidation following endurance training in the glycogen-depleted state. However, it should be noted that Mortensen et al. (68) showed that training in a low-glycogen state did not alter the expression of PGC-1α, PGC-1β, or PGC-1α-related coactivator. It is not overly surprising that PGC-1α mRNA is not changed after training, where a new steady state has been achieved. It is after acute exercise where the low-glycogen state would be expected to increase PGC-1α activity to a greater extent. In fact, Mathai et al. (64) showed that PGC-1α protein increased in direct proportion with the decrease in glycogen following acute exercise. However, Robinson et al. (79) did not see an increase in PGC-1α expression or mitochondrial protein synthesis within the first 5 h after a 1-h infusion of isoproterenol. However, since isoproterenol is not a specific β-agonist (isoproterenol also activates α-adrenergic, and this can antagonize β-activation), whether catecholamines can acutely regulate PGC-1α in humans remains to be determined.

Given the water content associated with glycogen, another potential "rheostat" function of glycogen within the cell may be to influence cellular osmotic pressure. Changes in muscle osmotic pressure are extremely difficult to measure in vivo. As a result, the effect of glycogen on osmotic tension in muscle is not clear in vivo. In vitro, where the osmolality of media can be changed and the effect on muscle glycogen can be measured directly, there are data to suggest that the amount of glycogen in a muscle can regulate osmotic tension (60). When the osmotic tension in the media is changed, cells respond in such a way as to equilibrate the osmolality inside the cell with that outside the cell. In muscle cells, decreasing the osmotic pressure in the media results in the synthesis of more glycogen in an effort to decrease muscular osmolality, whereas glycogen breakdown down occurs in hyperosmotic conditions in an attempt to increase osmolality in the muscle (60). Both of these responses are independent of changes in the rate of glucose transport. These data suggest that glycogen breakdown increases osmotic pressure within muscle. Mechanistically, hyperosmotic cellular stress is known to increase the activity of p38 MAPK (86). In glycogen-depleted muscle, p38 MAPK activity in the nucleus increases (16), suggesting that exercising with lower muscle glycogen could increase MAPK activation and drive skeletal muscle adaptive responses. One target of p38 is PGC-1α. The γ-subunit of p38 (p38γ) is required for PGC-1α induction and mitochondrial adaptation to endurance exercise (75). This makes p38γ the only protein known to prevent endurance adaptation in muscle, and therefore, the regulation and function of p38γ should be a major focus of endurance research. Another target of p38 is the myokine interleukin 6 (IL-6) (16). Keller et al. (54) demonstrated that plasma IL-6 protein increased 16-fold during exercise in a glycogen-depleted state compared with a 10-fold induction during normal glycogen conditions, whereas the IL-6 mRNA increased 100- and 30-fold, respectively. However, it needs to be mentioned that subsequent studies have shown that the IL-6 receptor is not influenced by glycogen content (53). One of the potential targets of IL-6 in muscle is AMPK (61, 80), and IL-6 knockout mice have dramatically reduced AMPK Thr^{172} (−50%) and acetyl-CoA carboxylase (ACC)-β Ser^{221} (−90%) phosphorylation in response to 60 min of swimming exercise (55), suggesting that glycogen depletion not only has direct effects on AMPK activation but, through cell stress and myokines, may have indirect effects on AMPK activity as well. It would be extremely interesting to determine whether the positive effects of glycogen depletion would be lost in these animals. Other myokines such as IL-8 are also increased during exercise in a low-glycogen state (16), suggesting that reduced glycogen may result in an altered myokine profile that may collectively serve to regulate skeletal muscle adaptation.

Glycogen-Sensitive Transcription Factors May Mediate the Adaptive Response

Exercise in a glycogen-depleted state dramatically alters the transcriptional profile in skeletal muscle (74). Although considerable attention has been directed toward coactivators such as PGC-1α, there is an emerging subset of transcription factors that appear capable of translating glycogen levels to altered gene expression. Although none of these transcription factors are known to regulate metabolism in response to differing levels of glycogen in skeletal muscle, their role in other tissues warrants discussion and investigation in this process. One such transcription factor is the carbohydrate response element-binding protein (ChREBP), a glucose-sensitive basic helix-loop-helix leucine zipper transcription factor that is highly expressed in liver, fat, and skeletal muscle (48). When glucose rises, ChREBP localizes in the nucleus, where it binds carbohydrate response elements (E boxes) in promoter regions of genes involved in metabolic regulation. In the liver, known ChREBP targets include pyruvate kinase, fatty acid synthase, and ACC (48). ChREBP nuclear localization and carbohydrate response element binding appear to be sensitive to cellular energy status, since energy depletion signals such as increased catecholamines or an increase in the AMP/ATP ratio lead to PKA- and AMPK-mediated phosphorylation and suppression of ChREBP activity (48). Recently, Dentin et al. (22) demonstrated that hepatic ChREBP activity was negatively regulated by polyunsatured fatty acids, regardless of chain length. Interestingly, this observation suggests that ChREBP, via regulation of glycolysis, may be an important signaling intermediate that allows rapid interchange between glucose and lipid metabolism (52). Ablation of the ChREBP gene in mice results in increased liver glycogen and decreased plasma FFA (48). The decrease in plasma FFA is possibly due to a shift from lipogenesis to lipolysis in the adipose tissue of these animals, resulting in decreased adipose depots. Intriguingly, the skeletal muscle of these animals has yet to be analyzed. In genetically intact animals, the increase in catecholamines and metabolic stress associated with glycogen depletion should have a similar effect, decreasing the activity of ChREBP, shifting the body from a lipogenic to a lipolytic state, and increasing plasma FFA (49). In one of the first reports to study the role of ChREBP in skeletal muscle, Hanke et al. (38) found that the upregulation of glycolytic/fast myosin in C_2C_{12} myotubes following a shift from low- to high-glucose media was partly dependent on ChREBP. Collectively, these data suggest that energy depletion or glucose restriction leads to suppression of ChREBP activity and a coordinated increase in lipid metabolism. However, the role of ChREBP in the regulation of skeletal muscle adaptations with different levels/localization of glycogen has yet to be addressed experimentally.

In addition to ChREBP, alteration in cellular nutrient availability has also been reported to alter the activity of the sterol response element-binding protein (SREBP) family, a group of endoplasmic reticulum-bound basic helix-loop-helix leucine zipper transcription factors that regulate the expression of a large subset of genes involved in lipid and cholesterol synthesis and utilization (51). To date, three isoforms of SREBP proteins have been characterized, termed SREBP-1a, SREBP-1c, and SREBP-2 (51). SREBP-1a appears capable of activating all SREBP targets via binding of sterol response elements within specific promoters. In contrast, SREBP-1c appears to be selective for genes involved in fatty acid synthesis, and SREBP-2 activates a program of cholesterol synthesis (28). SREBP activity is altered in skeletal muscle in response to acute and chronic exercise (69), acute fasting (10), and prolonged calorie restriction (69). In addition, SREBP activity may be associated with alterations in IMTG content and IMTG breakdown in skeletal muscle (69). SREBPs are potently activated by insulin, and their phosphorylation inhibits their transcriptional activity. Kinases known to suppress SREBP function include the extracellular receptor kinase 1/2, PKA, and glycogen synthase kinase-3β (52). In addition, n-3 and n-6 polyunsatured fatty acids suppress SREBP activity and nuclear abundance (52) apparently via increasing 26S proteosome-mediated ubiquitination of SREBP. The fact that factors associated with energy depletion reduce SREBP function (synthesis of IMTG) has led a number of investigators to speculate that the increased IMTG breakdown during exercise in a low-glycogen state may be associated with reduced SREBP function, thus switching IMTG turnover in favor of net breakdown. However, this has yet to be tested.

Heat shock protein 72 (HSP72) has also been suggested to regulate gene transcription in response to altered cellular substrate flux. Febbraio and Koukoulas (24) were the first to demonstrate that HSP72 activation paralleled muscle glycogen depletion in human skeletal muscle during prolonged endurance exercise. In a subsequent study, Febbraio et al. (27) showed that HSP72 activation following concentric exercise occurred only in a glycogen-depleted state, indicating that glycogen directly regulates HSP72 activity. Furthermore, the same group showed that increased glucose availability suppressed systemic HSP72 release following exercise (25) and that IL-6 could be the mechanistic link to increased HSP72 expression (26). This purported mechanism of activation is important because IL-6 gene expression and plasma abundance have been reported to be increased in a glycogen-depleted state postexercise compared with exercise in a normal glycogen state (53). Overexpression of HSP72 in skeletal muscle protects mice from high-fat diet-induced obesity, increases insulin sensitivity, and increases the enzyme activity of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase (18). Collectively, these data support the hypothesis that HSP72 could potentially convey some of the adaptive responses reported in a low-glycogen state.

Posttranslation Modifications Convey the Cellular Environment to Altered Protein Function

Cellular energy stress is also emerging as a key regulator of posttranslation modifications in skeletal muscle. As discussed above, considerable attention has been given to the regulation of protein phosphorylation by glycogen content, whereas recent research also suggests that substrate provision may also influence alternate posttranslation modifications such as lysine acetylation (the addition of acetyl groups to lysine residues) and *O*-GlcNAcylation (the addition of oligosaccharide groups to proteins).

A role for acetylation in the regulation of gene transcription was first suggested by Allfrey et al. (3), who demonstrated that RNA synthesis may be regulated by the addition of acetyl groups to core histone tails. Recently, two independent studies demonstrated that almost every enzyme involved in glycolysis, gluconeogenesis, fatty acid oxidation, glycogen metabolism, and the TCA cycle is acetylated and that the levels of acetylation varied when substrate flux through these pathways was manipulated (96, 107). To date, 2,200 proteins have been shown to be differentially acetylated (35). Lysine acetylation appears to shunt metabolism between metabolic pathways, since differential acetylation increased the activity of some enzymes and blunted the activity of others. Importantly, the activities of proteins that add (lysine acetyl transferases) or remove (deacetylases) acetyl moieties to lysine residues on proteins are directly regulated by cellular energy status (36). Lysine acetyl transferases appear to be active when substrate supply is high since substrate excess results in an increase in cellular acetyl-CoA, the substrate for acetyltransferase activity (21). In contrast, reduced cellular energy status increases cellular NAD^+ and activates members of the sirtuin (SIRT) deacetyase family in skeletal muscle (29). SIRT1 has been implicated in a number of fundamental cellular processes (100a). It is currently unknown whether SIRT1 activity is directly related to cellular glycogen content; however, exercise-induced glycogen depletion certainly occurs in parallel with increased SIRT1 activity (15). Whether this is causal or indirect in relation to glycogen content remains to be determined.

The role of *O*-GlcNAcylation in skeletal muscle is poorly understood (14). In a manner similar to acetylation, glycosylation appears to work in unison with phosphorylation to alter protein and enzyme activity (14). Importantly, glucose concentrations regulate glycosylation, providing the substrate for the hexosamine biosynthetic pathway (19). Key kinases for skeletal muscle metabolic adaptation (PKA, PKC, and p38 MAPK) have been shown to be glycosylated (106), and this modification appears to, in parallel with phosphorylation, alter metabolic function. Nearly 1,000 *O*-GlcNAc-modified proteins have been characterized to date (14); however, the physiological relevance of this process has yet to be determined (106). Given that glucose availability is known to alter enzyme activity, protein function, and substrate utilization, future investigation into the role of glucose-driven *O*-GlcNac modification is clearly an important avenue of research.

Future Directions and Practical Applications

We have tried to summarize some of the recent advances in the field of glycogen metabolism, but there are a number of questions that remained unanswered. The majority of the glycoproteomic data that we discussed was related to hepatic glycogen in a basal state. It will be fundamentally important to examine whether a similar glycoproteome exists in skeletal muscle or whether different proteins interact with glycogen in muscle. Furthermore, determining whether depletion of glycogen in skeletal muscle alters protein association with the glycogen granule and whether this can contribute to the im-

provement in fat oxidation following training in the low-glycogen state is a key question. Finally, once these protein groups have been determined, biochemical analysis will be required to examine how altering the glycogen-protein interaction affects individual protein/enzyme activity and function.

The hypothesis that manipulating glycogen can optimize training adaptations is relatively new, and as a result there are a number of important questions that remain to be answered. Mechanistically, we need to determine whether individual molecular targets such as PPARα/δ, AMPK, and PGC-1α mediate the improvement in fatty acid oxidation following low-glycogen training or whether changes in combinations of these factors, as well as many others, are required for a concerted adaptive response. If individual proteins are identified then the endogenous substrates that target and activate these proteins during glycogen depletion potentially hold great relevance for understanding skeletal muscle adaptation to exercise.

With regard to performing exercise in a glycogen-depleted state, it is still unknown whether a specific threshold exists at which point glycogen depletion increases cellular signaling. Given the decrements in force production with glycogen depletion (45, 103), understanding the trade-off between performance and signaling could be extremely beneficial in designing exercise regimes to maximize the "pro-signaling" environment initiated by glycogen depletion (73). On a similar theme, determining whether nutritional strategies could be used to alleviate the decline in power production or further amplify the signaling environment observed during exercise in a glycogen-depleted state is also an important, underinvestigated area of research (73).

Finally, given the recent suggestion that glycogen structure may be altered in rodent models of type 2 diabetes (92) and that glycogen depletion is important for the beneficial effects of exercise training in obese individuals (83), research examining the structure and partitioning of glycogen in clinical models of substrate excess and insulin resistance could potentially yield important answers regarding pathological substrate metabolism. With all of these questions remaining, the great history of glycogen research has many more chapters before we can close the book on glycogen in skeletal muscle.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.P., M.H., and K.B. drafted the manuscript; A.P., M.H., and K.B. edited and revised the manuscript; A.P., M.H., and K.B. approved the final version of the manuscript; K.B. prepared the figure.

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