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

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Abstract

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 towards fat oxidation, the increase in catecholamines, and osmotic stress. Even though our understanding of the role of glycogen in response to training has expanded exponentially over the last 5 years, there are still many questions remaining as to how stored carbohydrate affects the muscular adaptation to exercise.

Introduction

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 over 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 Bergstrom, Hultman, and Saltin. 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 re-synthesis was enhanced following glycogen depletion (super-compensation) (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, Bergstrom *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 specifically interact 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 post-translational 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 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 significantly affecting cellular osmolarity (66). Approximately 400mM of glucose can be stored in 0.01µM of 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 8000nm^3 (33). Indeed this branching design is critical, as 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. Even though 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: (i) the intermyofibrillar space, (ii) the intramyofibrillar space and (iii) 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 at present 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 (SERCA). The role of the subsarcolemmal glycogen fraction in fatigue is less clear at present, 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 (i) involved directly in the generation or regulation of the glycogen granule, or (ii) 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).

The glycogen proteome, those proteins that directly interact with glycogen, was recently determined from rat and mouse liver (88). The 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 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 a αβγ heterotrimer with multiple genes encoding each of the subunits (94). AMPK activity appears to be regulated by three fundamental processes, (i) binding of AMP, ADP, or ATP to the γ-subunit (94), (ii) phosphorylation of the catalytic α-subunit by a number of upstream kinases (90) and (iii) a glycogen-binding domain (GBD) located on the β-subunit (45, 76, 77). Whereas regulation 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 recently been explored 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 the 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 AMPK β-2^{Thr101} motif within the CBM reduced the affinity of AMPK for single α-1-6 branched oligosaccharides by 3-fold. Inserting the Thr^{101} motif into the AMPK β-1 subunit resulted in an increase in glycogen binding, confirming the functionality of the domain (58). However, whether the CBM can regulate AMPK signalling has yet to be determined.

As would be suggested by the effect of glycogen on AMPK activity, both the basal and post-exercise activity of AMPK-α2 is higher in the glycogen-depleted state (103). The ingestion of sufficient glucose to spare glycogen attenuates AMPK activation ~50% compared to 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), AMPK-α2 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 AMPK-α2 and subsequent increases in GLUT4 mRNA expression. Yeo et al. (104) also found that AMPK^{Thr172} 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 minutes of exercise in a glycogen depleted state. This led Steensberg and colleagues (89) 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 amongst the first to directly test the effect of skeletal muscle glycogen content on training adaptations. To achieve this, the authors employed an elegant contralateral leg-kicking model in which one leg trains twice a day, every other day (low glycogen), compared to 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 and colleagues demonstrated that 10-weeks of training with low muscle glycogen increased endurance (time to exhaustion), oxidative capacity (citrate synthase (CS) activity), and tended to increase 3-hydroxyacyl-CoA dehydrogenase activity (β-HAD) 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 Hansen study, the subjects performed 6 training bouts a week. However, in these studies half of the training bouts were long steady state rides at 70% VO_2max and half were high-intensity interval workouts. In

the high glycogen groups, the athletes exercised everyday alternating between steady state and HIT. In the low glycogen groups, the subjects trained every other day performing the HIT in a low glycogen state one hour 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-week training period, 60-minute time trial performance improved to the same extent in the low and high glycogen groups, indicating that relative to their exercise intensity the low glycogen group showed a greater adaptation. More interestingly, during steady state exercise at 70% VO_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 towards fatty acid oxidation, there was glycogen sparing and a greater increase in succinate dehydrogenase (SDH) and β-HAD 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 pro-adaptive responses induced during these twice a day, every other day, training protocols are due to alterations in an altered cellular environment which ultimate leads to enhanced signalling, or simply due to having a greater load and increased recovery period on each training day. If we consider the first scenario, examination of exercise studies that have directly or indirectly manipulated glycogen content have showed that exercise in a low glycogen environment is associated with: (i) elevated plasma free fatty acids (FFA); (ii) increased sympathetic nervous system activity; (iii) hyperosmotic stress (which results in elevated cellular stress) due to the loss of water

200 associated with glycogen; and (iv) increased myokine production (Figure 1). The potential
201 effects of these changes to the cellular milieu will be discussed below.

202 Exercise in a glycogen depleted state leads to increased whole body and skeletal muscle
203 lipid metabolism. One of the reasons for this shift is the liberation of free fatty acids from
204 adipose and intramuscular storage sites. The increased circulating FFA is important as it serves
205 two roles. First, as substrates for β-oxidation in the mitochondria (55), and second as signaling
206 intermediates for transcription factors and nuclear receptors that regulate the proteins
207 involved in the transport and breakdown of lipids (55). Understanding the molecular crosstalk
208 between the adipocyte and myocyte is an area of intense investigation. Two proteins that
209 appear to be important in the initial breakdown of lipid droplets in both adipose and muscle are
210 adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL). The complex regulation
211 of these proteins has been expertly reviewed elsewhere (99), however the action of these
212 proteins in the hydrolysis of lipid stores is important in the context of this review, as these
213 liberated fatty acids can serve as endogenous ligands for the peroxisome proiferator activated
214 receptor (PPAR) nuclear receptors alpha and beta/delta (PPARα/δ) (Figure 1).

215 There is limited information regarding the role of PPARδ in skeletal muscle metabolic
216 regulation. PPARδ is the most abundant PPAR in skeletal muscle and has been shown to be
217 enriched in oxidative type I fibers (13). Given the association of PPARδ expression with skeletal
218 muscle oxidative capacity, it is not surprising that both acute (62, 98) and chronic (30, 81)
219 exercise increase PPARδ mRNA expression in both rodent and human skeletal muscle. These
220 observations have therefore 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 (CPT-1)(32); 2) forkhead box O1 (FoxO1) (70); 3) fatty acid translocase (FAT/CD36) (43); and 4) hormone-sensitive ligase (HSL) (87). Fyffe and coworkers 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 (31). However, simply treating C2C12 myotubes with long-chain FFA failed to increase PPARδ expression (44). In contrast, Kleiner and colleagues demonstrated that in primary mouse myoblasts the PPARδ synthetic ligand GW501516 increased fatty acid oxidation in a PPARδ-dependent manner via up-regulation of key lipid target genes (57). 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. Further, 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.

As 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). Even though it is not clear how these sites regulate HSL activity, increased HSL activity drives lipolysis

both in adipose tissue and skeletal muscle. The result is the liberation of free fatty acids 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 co-activator, peroxisome proliferator activated receptor 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. Further, 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 PRC. 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. showed PGC-1α protein increased in direct proportion with the decrease in glycogen following acute exercise (64). However, Robinson et al (79) did not see an increase in PGC-1α expression or mitochondrial protein synthesis within the first 5 hours after a one hour 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 directly be measured, there is 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 osmolality, whereas glycogen is broken 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

could drive skeletal muscle adaptive responses. One target of p38 is PGC-1α. The gamma 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, while the IL-6 mRNA increased by 100 and 30-fold, respectively. It needs to be mentioned however, 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 knock-out mice have dramatically reduced $AMPK^{Thr172}$ (-50%) and $ACC\beta^{Ser221}$ (-90%) phosphorylation in response to 60min 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 which 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). Even though considerable attention has been directed towards co-

activators such as PGC-1α, there is an emerging subset of transcription factors that appear capable of translating glycogen levels to altered gene expression. Even though 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 (TF) is the carbohydrate response element binding protein (ChREBP), a glucose sensitive basic helix-loop-helix leucine zipper (bHLH-LZ) 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 (ChoRE, E boxes) in promoter regions of genes involved in metabolic regulation. In the liver, known ChREBP targets include pyruvate kinase (PK), fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) (48). ChREBP nuclear localization and ChoRE binding appears to be sensitive to cellular energy status, as energy depletion signals, such as increased catecholamines or an increase in the AMP to ATP ratio lead to PKA and AMPK mediated phosphorylation and suppression of ChREBP activity (48). Recently, Dentin and colleagues (22) demonstrated that hepatic ChREBP activity was negatively regulated by polyunsatured fatty acids (PUFAs), 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 anayzed. 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 C2C12 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 bHLH-LZ 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 (SREs) within specific promoters. In contrast, SREBP-1c appears 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

(ERK1/2), PKA and glycogen synthase kinase-3β (GSK-3β) (52). In addition, n-3 and n-6 PUFAs 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 only occurred in a glycogen depleted state, indicating that glycogen directly regulates HSP72 activity. Further, 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 as IL-6 gene expression and plasma abundance have been reported to be increased in a glycogen depleted state post-exercise compared with exercise in a normal glycogen state (53)**.** Over-expression 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 B-HAD (18). Collectively, this data supports the hypothesis that HSP72 could potentially convey some of the adaptive responses reported in a low glycogen state.

Post-translation modifications (PTM) convey the cellular environment to altered protein function

Cellular energy stress is also emerging as a key regulator of PTM 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 PTM 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 and colleagues who demonstrated that RNA synthesis may be regulated by the addition of acetyl groups to core histone tails (3). Recently, two independent studies demonstrated that almost every enzyme involved in glycolysis, gluconeogenesis, fatty acid oxidation, glycogen metabolism, and the TCA cycle are acetylated and that the levels of acetylation varied when substrate flux through these pathways was manipulated (96, 107). To date, 2200 proteins have been shown to be differentially acetylated (35). Lysine acetylation appears to shunt metabolism between metabolic pathways, as differential acetylation increased the activity of some enzymes, and blunted the activity of others. Importantly, the activity of proteins that add (lysine acetyl transferases; KAT) or remove (deacetylases; DAC) acetyl moieties to lysine residues on proteins are directly regulated by cellular energy status (36). KATs appear to be active when substrate supply is high, as substrate excess results in an increase in cellular acetyl-CoA, the substrate for increased 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 (reviewed by Schenk et al in this review series). 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 direct in relation to glycogen content remains to be determined.

The role of *O*-GlcNAcylation in skeletal muscle is poorly understood (14). In a similar manner 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 1000 *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. Further, determining whether depletion of glycogen in skeletal muscle alters protein association with the glycogen granule and whether this can contribute to the improvement 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 effects 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, under-investigated 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.

Figure Legend

Figure 1. Graphic representation of some of the signalling events that are differentially activated by exercise in either a (A) high glycogen or (B) low glycogen state. In a low glycogen state (B), the greater catecholamine response results in the activation of protein kinase A (PKA) and the phosphorylation and removal of the carbohydrate response element binding protein (ChREBP) and the sterol response element-binding protein (SREBP) from the nucleus. In addition, exercise in a low glycogen state leads to the phosphorylation and activation of hormone sensitive lipase (HSL), adipose triglyceride lipase (ATGL), and the activation of peroxisome proliferator activated receptor coactivator-1α (PGC-1α). The increase in circulating fatty acids (FA) and the breakdown of intramuscular triglycerides (IMTG) bind to and activate the peroxisome proiferator activated receptor (PPAR) delta resulting in an increase in the synthesis of mRNA associated with greater fat oxidation such as carnitine palmitoyltransferase (CPT-1), fatty acid 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. Lastly, the osmotic stress associated with a rapid change in glycogen content can activate the mitogen activated protein kinases (MAPK) such as p38, which can phosphorylate and activate PGC-1α. Together, these alterations in muscle signaling result in improved fatty acid utilization that ultimately results in glycogen sparing during steady state exercise.

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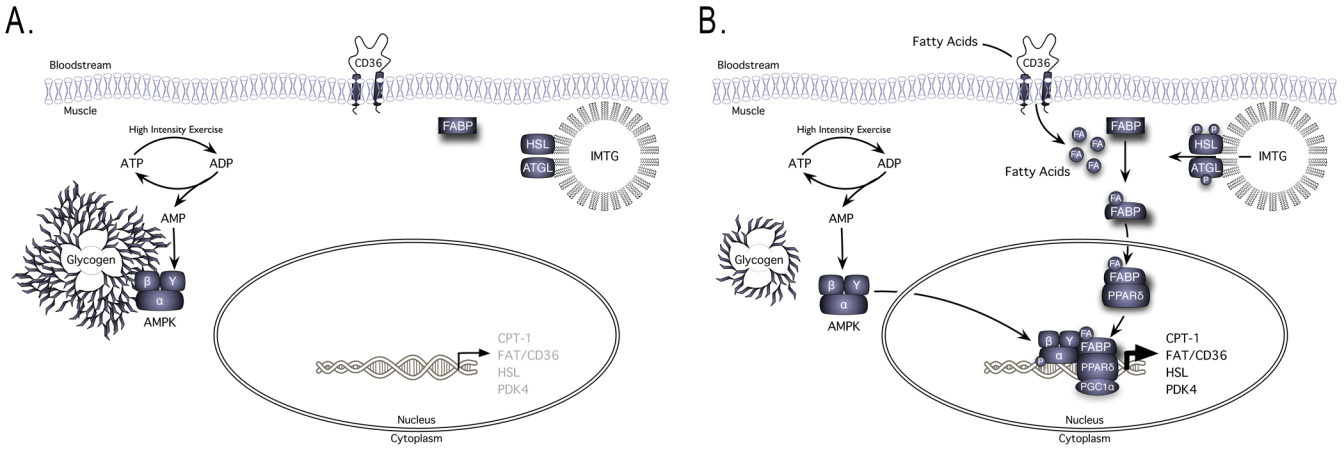
A.
Bloodstream
Muscle
CD36
FABP
HSL
ATGL
IMTG
High Intensity Exercise
ATP
ADP
AMP
Glycogen
β
γ
α
AMPK
CPT-1
FAT/CD36
HSL
PDK4
Nucleus
Cytoplasm
B.
Fatty Acids
Bloodstream
Muscle
CD36
FABP
Fatty Acids
FA
P
HSL
ATGL
IMTG
High Intensity Exercise
ATP
ADP
AMP
Glycogen
β
γ
α
AMPK
PPARδ
PGC1α
CPT-1
FAT/CD36
HSL
PDK4
Nucleus
Cytoplasm

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

Glycogen Metabolism
Glycogenin
Glycogen-branching enzyme
Glycogen debranching enzyme
Glycogen synthase
Glycogen phosphorylase
Protein phosphatase 1-α 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 α-1
40S ribosomal protein S18
60 kDa heat shock protein
Other Functions
Glucose-regulated protein 78 (BiP)
Glutathione S-transferase
Catalase