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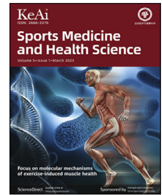
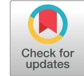


Review

The role of glycogen phosphorylase in glycogen biogenesis in skeletal muscle after exercise



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ABSTRACT

Initially it was believed that phosphorylase was responsible for both glycogen breakdown and synthesis in the living cell. The discovery of glycogen synthase and McArdle's disease (lack of phosphorylase activity), together with the high P_i/glucose 1-P ratio in skeletal muscle, demonstrated that glycogen synthesis could not be attributed to reversal of the phosphorylase reaction. Rather, glycogen synthesis was attributable solely to the activity of glycogen synthase, subsequent to the transport of glucose into the cell. However, the well-established observation that phosphorylase was inactivated (i.e., dephosphorylated) during the initial recovery period after prior exercise, when the rate of glycogen accumulation is highest and independent of insulin, suggested that phosphorylase could play an active role in glycogen accumulation. But the quantitative contribution of phosphorylase inactivation was not established until recently, when studying isolated murine muscle preparations during recovery from repeated contractions at temperatures ranging from 25 to 35 °C. Thus, in both slow-twitch, oxidative and fast-twitch, glycolytic muscles, inactivation of phosphorylase accounted for 45%–75% of glycogen accumulation during the initial hours of recovery following repeated contractions. Such data indicate that phosphorylase inactivation may be the most important mechanism for glycogen accumulation under defined conditions. These results support the initial belief that phosphorylase plays a quantitative role in glycogen formation in the living cell. However, the mechanism is not via activation of phosphorylase, but rather via inactivation of the enzyme.

Introduction

Glycogen is a branched, glucose polymer and the storage form of glucose, particularly in skeletal muscle and liver. The importance of glycogen as an energy substrate during many, if not most, forms of physical exercise is well established.[1] Additionally, glycogen plays a role in sarcoplasmic reticulum (SR) dependent Ca^{2+} release, muscle excitability, K^+ homeostasis, control of enzyme activities, gene expression, translational and post-translational processes, and has also been suggested to serve as a trap for inorganic phosphate (P_i).[2-5] Particularly relevant with respect to muscle performance is the observation that glycogen availability is critical for delaying fatigue during exercise in humans at exercise intensities corresponding to 60%–80% of maximal oxygen uptake (VO_2max).[6,7] Glycogen availability is also important for muscle performance during repeated contractions in isolated rodent muscle preparations under defined conditions.[8,9] During prolonged submaximal exercise in humans, glycogen is degraded and can reach very low levels or even depletion.[10-12] It follows that during recovery from exercise, glycogen must be resynthesized to ensure optimal performance during subsequent bouts of exercise. In this brief review, the biochemical basis for the accumulation of glycogen in skeletal muscle after exercise/repeated contractions will be described.

Enzymes of glycogen metabolism

Glycogen breakdown involves phosphorolysis as follows: $Glycogen_n + P_i \rightarrow Glycogen_{n-1} + Glucose\ 1\text{-}P$, where n reflects the number of glucose residues. The reaction is catalyzed by glycogen phosphorylase, which, together with debranching enzyme, results in full degradation of the glucose polymer. Phosphorylase is rate-limiting for glycogenolysis, and the reaction is considered to be irreversible in the cell. De-novo glycogen synthesis is believed to begin with the self-glucosylating protein, glycogenin, although questions remain regarding the true function of this protein.[13] Glycogenin uses uridine diphosphate glucose (UDPG) as a glucosyl donor and auto-glucosylates with ~10 glucose residues ($Glycogenin_0 + UDPG \rightarrow Glycogenin_{10} + UDP$), where 0 and 10 refer to the number of glucose residues before and after auto-glucosylation. Thereafter, glucosylated glycogenin can serve as a substrate for glycogen synthase (GS), which, in the presence of UDPG and, together with branching enzyme, forms mature (branched) glycogen ($Glycogen_n + UDPG \rightarrow Glycogen_{n+1} + UDP$). In practice, de-novo glycogen synthesis is

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Abbreviation list

AMPK	AMP-dependent protein kinase
EDL	Extensor digitorum longus
GS	Glycogen synthase
P_i	Inorganic phosphate
PP	Protein phosphatase
UDPG	Uridine diphosphate glucose
$\dot{V}O_2max$	Maximal oxygen uptake

uncommon as complete glycogen depletion is rarely achieved. Depending on the experimental conditions, either glucose transport or GS activity is considered to be rate-limiting for glycogen biogenesis in the living cell.[14] More detailed information on the regulation of the enzymes of glycogen metabolism in living muscle can be obtained elsewhere.[15]

Glycogen accumulation after exercise

The first study to demonstrate glycogen accumulation after exercise in human muscle involved one-legged cycling. The authors clearly demonstrated a marked accumulation of glycogen solely in the leg that underwent prior depletion during cycling. Not only was the initial muscle glycogen level restored, and even slightly surpassed, within 24 h, but by 3 days post-exercise, glycogen levels were ~twice as high vs. the basal state prior to the onset of exercise. These findings led to the conclusion that exercise with glycogen depletion enhances the accumulation of glycogen and this effect is localized to the previously active muscle.[10] Subsequent studies confirmed these findings and established several mechanisms to account for the process of glycogen accumulation following exercise, as well as the phenomenon of glycogen supercompensation (values exceeding the basal level). Based on human and animal studies, glycogen accumulation and supercompensation were attributed to activation of glucose transport, increased expression of GLUT1 as well as GLUT4 protein expression, activation of GS, as well as prolonged activation of an isoform (α1β2γ1) of AMP-dependent protein kinase (AMPK), which was suggested to enhance fatty acid oxidation and inhibit carbohydrate oxidation, thereby channeling more glucose toward glycogen synthesis.[16-19] As indicated above, the rate-limiting step can vary depending on the experimental conditions. For example, overexpression of GLUT1 protein in mouse skeletal muscle leads to marked increases in muscle glycogen levels in the absence of activation of GS, as judged by dephosphorylation of the enzyme.[20] Moreover, overexpression of hexokinase II protein on a GLUT1 overexpression background increases glycogen even further.[21] Similarly, overexpression of GS protein in mouse skeletal muscle leads to marked increases in muscle glycogen levels in the absence of increases of GLUT4 protein (in fact GLUT4 protein was significantly decreased).[22] Such data demonstrate the complexity of determining what reaction(s) is rate-limiting for glycogen accumulation and illustrate the importance of describing the conditions of study.

Accumulation of glycogen in human muscle after exercise occurs in two phases: 1. an initial rapid phase that is independent of insulin and enhanced by low glycogen levels; and 2. a subsequent slower, prolonged phase that is dependent on insulin.[23] Considering the importance of resynthesizing glycogen during recovery from exercise (see above), various strategies have been employed to accelerate and enhance the magnitude of this process. Ingestion of a carbohydrate rich diet after prior glycogen depletion has proven to be an effective approach.[6,10,16,24] In addition to provision of substrate for glycogen synthesis (glucose), such a diet will also stimulate insulin secretion, which will enhance both glucose transport and activation of GS. Another approach that has proven useful in enhancing glycogen accumulation during the first hours of recovery after exercise has been the addition of protein to carbohydrate supplements. This practice results in larger increases in plasma insulin, likely due to the synergistic effects of glucose and protein/amino acids on insulin secretion.[25] Yet another approach to increase muscle glycogen involves heating of muscle after glycogen-depleting exercise. This is illustrated by the observation that after substantial breakdown of glycogen in both thighs after exercise, subjects ingested carbohydrates at the end of exercise, as well as 2 h later. Biopsies were obtained at the end of exercise and 4 h later for measurements of glycogen. After exercise, a heat pack was applied to one thigh, which resulted in an increase of muscle temperature by > 2 °C vs. the control thigh. Glycogen accumulation was significantly higher in the heated thigh.[26] The authors attributed the enhanced glycogen accumulation after heating primarily to a Q_{10} effect.

Heating and glycogen biogenesis

To study the mechanism whereby heating enhances glycogen accumulation during recovery, a study was performed on isolated mouse extensor digitorum longus (EDL, fast-twitch, glycolytic) muscle.[27] Muscles were stimulated electrically to perform repeated contractions at 25 °C until force declined to 40% of initial. Thereafter muscles recovered in medium containing 5.5 mM glucose at 25 °C for 120 min (control), or at 35 °C for 120 min, or at 35 °C for 60 min followed by 60 min at 25 °C.[27] Following, the 120 min of recovery, a second fatigue run was performed at 25°C in all 3 groups. In the control group, the number of contractions during the second fatigue run was slightly less than during the first run (92% ± 5%). Surprisingly, in the group that recovered for 120 min at 35 °C, the results in the second fatigue run were also similar to those observed in the first (98% ± 6%). If heating enhanced glycogen synthesis, then increased levels of glycogen and enhanced performance would be expected. Indeed, measurements of glycogen synthesis (^{14}C-glucose incorporation into glycogen) during recovery from the first fatigue run demonstrated a 1.7-fold higher rate at 35 °C than at 25 °C.[27] However, glycogen contents were similar after 120 min recovery at the low and high temperature. Decreasing recovery duration at 35 °C to 60 min followed by an additional 60 min at 25 °C, resulted in a small but significantly enhanced performance during the second fatigue run (110% ± 2%), but, again, there were no detectable increases in glycogen levels vs. control conditions (120 min at 25 °C). Another noteworthy finding was that following recovery at 35 °C (both 120 and 60 min), half relaxation time during the second fatigue run was significantly slower vs. the first run. This is a well-established phenomenon of fatigued muscles.[27] These experiments demonstrated that recovery at the higher temperature resulted in some unexpected negative effects on muscle function/metabolism. Moreover, the findings on glycogen levels in isolated mouse muscle were in apparent contrast to the afore-mentioned results where local heating resulted in increased glycogen levels during recovery after exercise in humans.

Phosphorylase and glycogen biogenesis

Insofar as glycogen levels are dependent on two opposing reactions, glycogen synthesis and glycogen breakdown that are catalyzed, respectively, by GS and phosphorylase, the unexpected findings described above were investigated further. In the next study, marked glycogen degradation was achieved by using an intense stimulation paradigm (70 Hz, 100 ms trains, 2 trains/s for 10 min resulting in 1 200 contractions) at 25 °C in isolated EDL muscle.[28] This approach resulted in a decrease of glycogen to 15% of basal. Thereafter, muscles recovered at 25 °C for up to 240 min (control) or at 35 °C for 120 min followed by an additional 120 min at 25 °C. During recovery at 25 °C, glycogen synthesis (^{14}C-glucose incorporation into glycogen), increased about 2-fold vs. basal (i.e., at 25 °C), consistent with a Q_{10} value of ~2. However, during recovery at 35 °C, glycogen synthesis increased to a value > 3-fold higher than during basal, which was consistent with the earlier findings.[27] Measurements of glycogen content during recovery at 25 °C yielded values that

corresponded to ~75% of basal after 120 min and essentially baseline levels after 240 min. These findings were similar to those reported earlier under comparable conditions.[29] In contrast, after 120 min recovery at 35 °C, glycogen levels remained low and similar to the mean value observed at the end of repeated contractions. This finding could not be attributed to some form of irreversible muscle damage induced by the intense stimulation paradigm, as demonstrated by the observation that recovery of force was comparable after 120 min of recovery at the two temperatures (in fact force was increased at low frequencies following recovery at 35 °C vs. 25 °C).[28] Moreover, reversing temperature from 35 °C to 25 °C from 120 to 240 min of recovery resulted in a rapid rate of glycogen accumulation that was comparable to that observed during the first 120 min of recovery at 25 °C.

To examine the basis for these surprising findings, the activities of GS and phosphorylase were measured. Following repeated contractions, GS fractional activity increased markedly, reflecting dephosphorylation and activation of the enzyme.[28] However, following 120 min of recovery, the higher rate of glycogen synthesis at 35 °C vs. 25 °C could not be attributed to activation of GS either by dephosphorylation or by allosteric activation by glucose 6-P. Rather, the higher rate of glycogen synthesis was attributed primarily to a Q_{10} effect on GS. Measurements of phosphorylase activity demonstrated that immediately after 10 min of repeated contractions, there was little change in phosphorylase fractional activity (which reflects the phosphorylation state of the enzyme). Most likely phosphorylase phosphorylation (which reflects activation that can be independent of increases in AMP) occurred during the initial phase of repeated contractions and this was reversed as contractions continued.[15] Noteworthy, was the observation that phosphorylase fractional activity decreased markedly throughout the 240 min of recovery compared to baseline and the results were similar at both temperatures. These findings were not unique as they had been observed previously and served as a basis for the suggestion that phosphorylase inactivation can contribute to glycogen accumulation during recovery from exercise.[29-31] However, the quantitative significance of phosphorylase inactivation was not estimated in these studies.

Glycogen accumulation is dependent on the simultaneous rates of glycogen synthesis (estimated by the incorporation of radio labeled glucose into glycogen) and breakdown. By measuring glycogen accumulation and synthesis, it is possible to calculate the rate of degradation and thereby assess quantitatively the contribution of phosphorylase to glycogen accumulation. (Glycogen degradation can also be assessed with isotopic techniques.[32]) Such measurements and calculations were recently performed when studying isolated murine muscle preparations during recovery from repeated contractions at temperatures ranging from 25 to 35 °C. Thus, in both slow-twitch, oxidative and fast-twitch, glycolytic muscles, inactivation of phosphorylase accounted for 45%–75% of glycogen accumulation during the initial hours of recovery following repeated contractions.[28,33] Such data indicate that phosphorylase inactivation may be the most important mechanism for glycogen accumulation under defined conditions. Intuitively, this may appear difficult to understand considering that it is often assumed that phosphorylase is active during exercise and that GS is active during recovery from exercise. However, it should be stressed that the maximal activity of phosphorylase is usually more than 10-fold greater than that of GS when measured in vitro under optimal conditions.[15] And both enzymes can be activated or inactivated during exercise as well as recovery depending on the experimental conditions and control via various mechanisms, such as covalent phosphorylation, substrate availability and allosteric regulation. Thus phosphorylase is almost exclusively responsible for disappearance of glycogen during exercise with only a minor contribution of GS inhibition to this process.[15] Therefore, even if both enzymes would be fully activated during exercise, it is clear that phosphorylase would predominate in the control of net glycogen breakdown. In contrast, during recovery, phosphorylase can also be quantitatively the most important for glycogen accumulation. The reason for this is that the rate of glycogen synthesis/accumulation during recovery from exercise is extremely low compared with the rate of glycogenolysis/net breakdown during intense exercise/repeated contractions (often $< 1\%$).[15] Hence any small relative change in the activity of phosphorylase, either in the direction of activation or inhibition, will have a large absolute effect on the rate of glycogen accumulation.

The idea that phosphorylase inactivation is quantitatively important for glycogen accumulation appears to apply to conditions other than exercise as well. Thus, it is well known that exposure of isolated rat muscle preparations to insulin results in activation of GS, stimulation of glycogen synthesis and glycogen accumulation, but insignificant changes in phosphorylase fractional activity.[34-37] However, phosphorylase fractional activity in carefully dissected isolated muscle preparations is generally low and, therefore, it is difficult to detect a decrease after exposure to insulin. But by measuring the amount of phosphate bound to immunoprecipitated phosphorylase in isolated muscle it was found that the amount of phosphate associated with phosphorylase was decreased by 50% following exposure to insulin.[38] Using synthetic inhibitors of GS and phosphorylase, others have also demonstrated that inactivation of phosphorylase is an important component of insulin-mediated glycogen accumulation in hepatocytes.[39] Moreover, measurements of glycogen cycling (simultaneous synthesis and degradation) in isolated muscle using isotope techniques demonstrated that insulin exposure resulted in a 3-fold increase in glycogen synthesis, whereas glycogenolysis was inhibited ~7-fold.[32] Such findings support the quantitative importance of phosphorylase inhibition (by dephosphorylation) in the accumulation of glycogen in skeletal muscle.

Biochemical basis for phosphorylase inactivation during recovery from exercise/repeated contractions

Phosphorylase is traditionally believed to be regulated by 3 mechanisms: 1. phosphorylation/dephosphorylation; 2. allosteric control; and 3. substrate availability.[40] Recently, other forms of covalent modification of phosphorylase have been described, including: acetylation, oxidation and nitration.[41-43] Acetylation of a lysine residue (470) on the liver phosphorylase isoform results in enhanced dephosphorylation (resulting in inactivation) of the enzyme by protein phosphatase 1 (PP1).[41] However, to the author's knowledge such a mechanism has not been described in the muscle phosphorylase isoform. Similarly, oxidation of critical cysteine residues in the brain phosphorylase isoform precludes AMP-dependent activation.[42] However, oxidation of the skeletal muscle isoform is minimal[42] and does not appear to play a role in control of phosphorylase and glycogenolysis during repeated contractions in isolated muscle preparations.[44] In contrast, nitration of tyrosine (613) of skeletal muscle phosphorylase results in potent inhibition of the purified enzyme,[43] as well as crude muscle extracts.[44] Indeed, exposure of isolated muscle preparations to supraphysiologic concentrations of peroxynitrite results in nitration of phosphorylase and inhibition of glycogenolysis during repeated contractions. However, the production of endogenous nitrating agents during repeated contractions does not result in detectable nitration of phosphorylase nor does it affect glycogenolysis.[44]

The major mechanism for phosphorylase inactivation (i.e., dephosphorylation) during recovery from exercise/repeated contractions likely depends on an increased ratio in the activities of phosphorylase phosphatase/kinase. Phosphorylase dephosphorylation is accounted for by the activity of PP, primarily PP1.[45] The activity of PP decreases by ~30% after intense isometric contraction to fatigue in humans (~60 s).[46] If the circulation is intact, PP1 activity increases by ~80% (vs. fatigue) within 5 min after the end of exercise,[46] which would contribute significantly to phosphorylase dephosphorylation. The activity of phosphorylase b kinase, which phosphorylates phosphorylase (the b form), decreases within seconds after contraction, likely because of the rapid sequestration of Ca^{2+} into the sarcoplasmic reticulum.[47,48] Indeed early studies by Cori and colleagues estimated that phosphorylase phosphatase activity exceed that of phosphorylase kinase after muscle contraction.[49] Of particular

significance here is the persistent inability to induce phosphorylation of phosphorylase (i.e., activation by converting the b form to the a form) by epinephrine or muscle contraction following a bout contractile activity.[50] The prolonged resistance to phosphorylation after exercise/repeated contractions may be related to a sustained activation of PP activity. Finally, under certain conditions (e.g., heating), an increase in substrate availability (P_i) can enhance phosphorylase activity and thereby limit glycogen accumulation.[28,33]

In this brief review, the quantitative importance of phosphorylase activity in determining the rate of glycogen accumulation following exercise has been discussed. When phosphorylase was originally discovered and characterized in the 1940s, it was believed that the enzyme was responsible for glycogen breakdown and glycogen synthesis in the living cell. However, studies in the 1950s clearly established that the process of glycogen synthesis in the living cell could not be accounted for by reversal of the phosphorylase reaction, but rather by the activation of GS. From the data presented and discussion in this review, it appears that the initial belief that phosphorylase plays a quantitative role in glycogen formation in the living cell was correct. However, the mechanism is not via activation of phosphorylase, but rather via inactivation of the enzyme.

Author's contribution

The author is solely responsible for all aspects of the manuscript.

Submission statement

The author has read and agrees with the manuscript content. While this manuscript is being reviewed for this journal, the manuscript will not be submitted elsewhere for review and publication.

Conflict of interest

The author has no direct or indirect interests that are in direct conflict with the conduction of the study.

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