

Regulation of glycogen synthase in skeletal muscle during exercise

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Abstract

Glycogen synthase (GS) catalyses the incorporation of uridine diphosphate-glucose into glycogen in skeletal muscle. In concert with the glucose transport step, GS activity is thought to be rate-limiting in the disposal of glucose as muscle glycogen. Glycogen synthase is regulated by both allosteric factors (primarily glucose 6-phosphate) and covalent modification by reversible phosphorylation and dephosphorylation leading to inactivation and activation of GS, respectively. Exercise activates both stimulatory and inhibitory regulators of GS and it is thought that the resultant activity of GS during exercise depends on the relative strength of opposing signals. However, the mechanisms by which exercise regulates GS activity are not fully understood. Glycogen breakdown, the G_M -protein phosphatase 1 complex and possibly cellular relocalization of GS may be considered important factors involved in the stimulation of GS activity during exercise, while adenosine monophosphate-activated protein kinase and plasma adrenaline (via protein kinase A) can be considered as essential for the exercise-induced inhibitory signals to GS.

Keywords adrenaline, AMP-activated protein kinase, glycogen, glycogen synthase kinase 3, protein phosphatase 1.

Once taken up in skeletal muscle, glucose is phosphorylated to glucose 6-phosphate, which is either directed towards glycolysis or glycogen synthesis. After a glycogen depleting stimulus, e.g. exercise or fasting, the rise in insulin induced by a carbohydrate-rich meal will direct a substantial part of the glucose towards repletion of the glycogen stores. This is brought about by an insulin-induced increase in the activity of glycogen synthase (GS), which is a catalyst of the incorporation of uridine diphosphate (UDP)-glucose into glycogen. Exercise increases the energy demand of the contracting muscle and a significant part of this demand for adenosine triphosphate (ATP) generation is met by speeding up glycogen breakdown. Although exercise is most often associated with a net breakdown of glycogen, mechanisms exist that promote the activity of GS in response to exercise in an insulin-independent manner. Before going into a discussion of the exercise regulation

of GS, a general description of the complex mechanisms involved in regulation GS activity is warranted.

Regulation of glycogen synthase activity

Glycogen synthase is regulated allosterically by glucose 6-phosphate as well as by reversible phosphorylation and dephosphorylation leading to inactivation and activation of GS, respectively. Thus, activation of GS may take place through inactivation of kinases phosphorylating GS and by activation of phosphatases dephosphorylating GS. Several of these upstream enzymes have been identified although their relative importance in the regulation of GS is not fully clarified. The significance of most of these enzymes is discussed in relation to exercise in the following sections of this review.

In general, phosphorylation decreases GS activity by causing an increase in the K_M for the substrate

UDP-glucose and an increase in the K_a for glucose 6-phosphate (Roach & Larner 1976, Roach *et al.* 1976). Besides allosteric activation of GS, glucose 6-phosphate also induces an increased and decreased susceptibility of GS to the action of GS phosphatases and protein kinase A (PKA), respectively (Lawrence & James 1984, Villar-Palasi 1991, 1994, 1995). It should be remembered that when measuring GS activity in a standard *in vitro* assay (Thomas *et al.* 1968) as applied in most studies the direct allosteric effect of G6P on GS *in vivo* is not picked up, whereas indirect effects of G6P on the susceptibility of GS to upstream enzymes may be. Thus, GS activity measurements are thought to reflect the effect of phosphorylation status on the enzyme activity.

Glycogen synthase is subject to phosphorylation at nine or more sites. Two sites, site 2 and 2a, corresponding to Ser⁷ and Ser¹⁰ on GS from rabbit skeletal muscle, are located near the NH₂-terminus. At least seven sites (sites 3a–c, 4, 5, 1a–b) are located in the COOH-terminal 100 residues of GS. Phosphorylation of site 2, 2a, 3a and b generally decreases the activity more than phosphorylation of the remaining sites that have minor or no effect on activity of GS (DePaoli-Roach *et al.* 1983, Poulter *et al.* 1988, Flotow & Roach 1989, Nakielny *et al.* 1991, Zhang *et al.* 1993). Notably, initial phosphorylation of site 5 by casein kinase (CK) 2 creates a recognition motif for GSK3, which then sequentially phosphorylates site 4, 3c, 3b and 3a (Fiol *et al.* 1987, Roach 1990). This phenomenon has been termed hierarchal phosphorylation (illustrated in Fig. 1), which has also been demonstrated for the NH₂-terminal sites of GS where modification of site 2 by PKA, AMPK or other kinases (reviewed by Roach 2002) is a prerequisite for phosphorylation of site 2a by CK 1 (Flotow & Roach 1989, Flotow *et al.*

1990). Thus, the extent of activation depends on the specific site phosphorylated and on the glucose 6-phosphate concentration (reviewed in Lawrence & Roach 1997, Roach 2002).

Exercise regulation of GS

Glycogen is an essential metabolic substrate during exercise even at fairly low work intensities (Karlsson & Saltin 1971). One clear example of this is the severe exercise intolerance present in patients with McArdle's disease characterized by absence of muscle glycogen breakdown due to deficiency of a functional skeletal muscle glycogen phosphorylase enzyme (McArdle 1951, Nielsen *et al.* 2002a). Repletion of the glycogen stores involves an increase in the activity of GS *after* exercise. However, it has been proposed that *during* exercise although glycogen is broken down, mechanisms exist that at the same time promote the activity of GS and in turn glycogen synthesis (Huang 1998, Nielsen *et al.* 2001, Shulman & Rothman 2001).

The regulation of GS by exercise is not dependent on the muscle insulin receptor (Wojtaszewski *et al.* 1999a) and muscle contraction regulation of GS is PI3-kinase independent (Wojtaszewski *et al.* 1999b, Sakamoto *et al.* 2002). These are observations that strongly suggest that insulin and exercise utilize different signalling pathways to activate GS, at least at the proximal level. Notably, the stimulatory effects of exercise and insulin are not always additive (Wojtaszewski *et al.* 1999a), suggesting that the two stimuli, at least in part, affect GS in the same way, e.g. the same phosphorylation sites.

Early studies in rodent (Danforth 1965, Piras & Staneloni 1969) and later studies in human skeletal muscle demonstrated that GS activity was *increased* in response to exercise (Bergstrom *et al.* 1972, Kochan *et al.* 1979, Bak & Pedersen 1990, Yan *et al.* 1993b, Wojtaszewski *et al.* 2001). However, in response to exercise conditions characterized by limited glycogen breakdown and/or high exercise intensities, GS activity has actually been observed to be *decreased* or unchanged during exercise and rapidly increased *after* the cessation of exercise (Chasiotis *et al.* 1982, 1983a, Chasiotis & Hultman 1985, Kida *et al.* 1989, Yan *et al.* 1992, 1993a, Katz & Raz 1995, Jiao *et al.* 1999, 2001). Taken together, there seems to be a consensus that exercise and insulin regulate GS activity differentially (Danforth 1965, Aschenbach *et al.* 2001, Nielsen *et al.* 2001, Suzuki *et al.* 2001) and as discussed in the following, we have put forward the hypothesis that GS is influenced by both stimulatory and inhibitory factors during exercise and the consequent effect of exercise on GS activity is a result of the relative strength of these opposing signals that may vary according to the exercise mode, intensity and duration.



Figure 1 Schematic model of glycogen synthase (GS). The enzyme is shown in cartoon form with indications of the different phosphorylation sites and the primary kinases thought to act on these sites. The serine residue numbers refer to the rabbit muscle GS enzyme. Hierarchal phosphorylation is indicated on site 2a and the sites 3–4. Site 2, 2a, 3a and 3b are the sites thought to be most important in the regulation of GS activity. Also indicated (G6P) is the region of GS responsible for the sensitivity to glucose 6-phosphate. The primary phosphatase thought to be responsible for dephosphorylation of all sites on GS is protein phosphatase 1 (not depicted). Refer to text for further detail and references. Adapted from Roach (2002) and Hanashiro & Roach (2002).

Muscle glycogen content

It has long been known that GS activity is closely coupled to the muscle glycogen content in both rodent (Danforth & Harvey 1964, Danforth 1965) and human skeletal muscle (Bergstrom *et al.* 1972), both in the resting state and after muscle contraction (Danforth 1965, Zachwieja *et al.* 1991, Nielsen *et al.* 2001). This inverse relationship is very tight and notably data-points from resting and contraction-stimulated muscle follow the same hyperbolic-shaped curve, whereas insulin and adrenaline-stimulation shift the curve up and down, respectively (Danforth 1965, Nielsen *et al.* 2001) as indicated in Figure 2. This strongly suggests that the increase in GS activity induced by muscle contraction is merely a result of the decreasing glycogen content and suggests that changes in glycogen content during exercise (and at rest) play an essential role in the regulation of GS. In other words, whichever signalling pathway activates GS during exercise it does not seem to have a glycogen-independent effect on GS. In support of such a mechanism is the observation that in patients with McArdle's disease, which are unable to break down glycogen due to deficiency of glycogen phosphorylase, GS is not activated after exercise in contrast to a significant activation of GS in the control group. These patients also have low resting activities of GS, most likely due to the exaggerated muscle glycogen stores (Nielsen *et al.* 2002a). Further evidence for glycogen breakdown being essential for exercise-induced

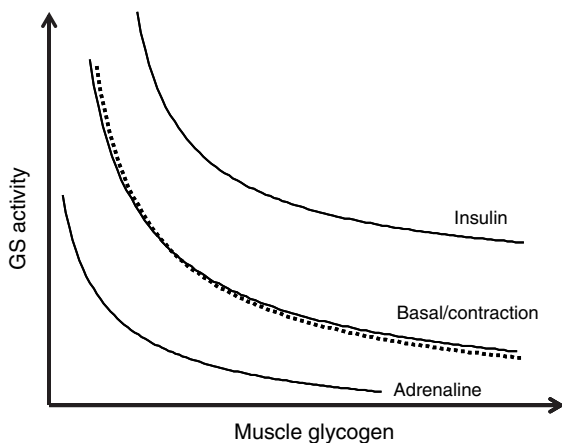


Figure 2 Relationship between the muscle glycogen concentration and the glycogen synthase (GS) activity. GS activity is strongly related to the muscle glycogen concentration in an inverse hyperbolic manner. Basal and contraction-stimulated muscle follow the same curve suggesting that the contraction-induced increase in GS activity is a result of glycogen breakdown. Insulin and adrenaline do not abolish the relationship between glycogen content and GS activity but shift the curve up and down, respectively. The figure is based on studies referred to in the text.

activation of GS is the observation that in healthy subjects with low muscle glycogen levels prior to exercise and no further net glycogen breakdown during exercise, GS is not increased over the resting level during exercise. In contrast, when muscle glycogen is elevated prior to exercise, glycogen is broken down during exercise and GS activity is increased above the pre-exercise level during exercise (unpublished data from our laboratory).

The mechanism underlying the relationship between GS activity and muscle glycogen content has not yet been revealed. An attractive hypothesis could be that changes in GS activity are related to the cellular localization of GS and its upstream effectors. In 3T3-L1 adipocytes, primary hepatocytes, C2C12 myotubes and COS-1 cells, GS is capable of translocation in response to various stimuli but clear differences in GS distribution exist between cell/tissue types (Fernandez-Novell *et al.* 1992, 1997, Baque *et al.* 1996, Brady *et al.* 1999, Ferrer *et al.* 2000, Jensen *et al.* 2000, Garcia-Rocha *et al.* 2001). Skeletal muscle with its contractile apparatus is functionally different from adipocytes and hepatocytes and notably, cytosolic GS is present in adipocytes but not in skeletal muscle, where cytosol is defined as the high-speed (>100 000 g) supernatant from non-detergent-treated homogenate (Brady *et al.* 1999, Nielsen *et al.* 2001). Therefore, extrapolation of results concerning GS and related enzymes from non-muscle cell types to the regulation of GS in skeletal muscle should be done cautiously. In skeletal muscle it has been demonstrated that GS is associated with glycogen particles (Meyer *et al.* 1970, Bergamini *et al.* 1977) but also with myofibrils (Moruzzi *et al.* 1980, Lane *et al.* 1989, Nielsen *et al.* 2001). Moruzzi *et al.* (1980) demonstrated that the recovery of total GS activity (a measure of GS protein content) in myofibrillar sediments increased in response to adrenaline administration *in vivo* and our experiments indicate that this is most likely due to the glycogen-depleting action of adrenaline (Nielsen *et al.* 2001). In the latter study, it was also demonstrated in fast-twitch muscle that some GS translocates from the glycogen-enriched fraction to the cytoskeleton when glycogen content in skeletal muscle decreases. Furthermore, GS protein content of a 2700 g pellet in fast-twitch muscle increased when glycogen was low but this was not the case in the slow-twitch soleus muscle (Nielsen *et al.* 2001). Moruzzi *et al.* (1980) were also able to show an effect of adrenaline on GS translocation in fast but not in slow-twitch muscle, suggesting that GS translocation to the cytoskeleton is muscle fibre-type specific.

A myofibrillar localization of GS may be explained by the finding that glycogenin is partly associated with the cytoskeleton protein actin (Baque *et al.* 1997), which constitutes a major part of the sarcomeric I-band.

Furthermore, Skurat *et al.* (2002) recently showed that GNIP (a novel glycogenin and GS interacting protein) interacts with desmin, another myofibrillar protein. The preferential cytoskeleton localization of GS when glycogen stores are depleted probably indicates that glycogen synthesis close to the contractile apparatus has a high priority. It is presumably advantageous to have the glycogen stores located close to the site of energy utilization especially because mitochondria also accumulate in this region (Eisenberg *et al.* 1974, Eisenberg & Kuda 1975). When glycogen stores near the contractile apparatus are filled up then further glycogen synthesis can occur in other regions of the cell and GS association with the myofibrils is relaxed. In the early stages of glycogen synthesis, glycogenin and GS are associated, but later they dissociate as synthase is thought to move to the outer branches of the glycogen particle (Smythe & Cohen 1991). If GS activity is high only when glycogenin and GS are bound to each other as suggested by *in vitro* studies (Pitcher *et al.* 1987, 1988), this could explain why GS activity decreases as the glycogen particle grows.

Protein phosphatase 1

Already Larner *et al.* (1967) speculated that both GS and a GS phosphatase are bound to glycogen in the cell and when the glycogen content is decreased during exercise GS and the phosphatase would be released from glycogen, enabling the phosphatase to activate GS. When glycogen levels increase again, GS activity would decrease because glycogen binding would inhibit the phosphatase. According to this hypothesis, glycogen-effects on phosphatase activity would play a major role in the inverse relationship between muscle glycogen levels and GS activity. Although some studies support the existence of such a mechanism (Villar-Palasi & Larner 1966, Villar-Palasi 1969, Mellgren & Coulson 1983) several others do not (Holmes & Mansour 1968, Martensen *et al.* 1973, Hubbard & Cohen 1989b, Bak & Pedersen 1990, Vardanis & Hudson 1991, Yan *et al.* 1992, Halse *et al.* 2001, Wojtaszewski *et al.* 2002). However, when evaluating these studies it should be considered that in assays of total phosphatase activity in homogenates or lysates of skeletal muscle changes in activity of targeted subpopulations of phosphatases are not picked up and possibly interactions between subunits important for the phosphatase activity are disrupted. Also as homogenization of the tissue is done without phosphatase inhibitors (because phosphatase activity is measured) any effect due to changes in phosphorylation of protein phosphatase 1 (PP1) catalytic and targeting subunits could be lost.

The primary phosphatase acting on the GS sites important for activity is the multi-substrate phosphatase

PP1 (Cohen 1989). The regulation of PP1 is complex and the reader is referred to recent comprehensive reviews on this topic (Newgard *et al.* 2000, Brady & Saltiel 2001). With respect to regulation of GS activity it should be mentioned that GS, glycogen phosphorylase and phosphorylase kinase are all to a variable extent bound directly to glycogen (Cohen 1978) but several lines of evidence indicate that these enzymes as well as PP1 are also bound to PP1-targeting subunits that themselves bind to glycogen and acts as molecular scaffolds thereby having a major impact on regulation of glycogen metabolism. One such targeting subunit that has received considerable attention is G_M (or RGI for glycogen-binding regulatory subunit) that is also able to bind to SR-membranes via a specific site distinct from the glycogen binding site (Tang *et al.* 1991, Hubbard & Cohen 1993). Binding of G_M to PP1 enhances dephosphorylation of GS, whereas glycogenolytic agents such as adrenaline cause phosphorylation of rabbit G_M at serine 67 (corresponding to serine 65 in the human isoform), and, in some reports, also serine 48 (corresponding to serine 46 in the human protein), resulting in dissociation and thus inactivation of the G_M -PP1 complex (Hiraga & Cohen 1986, Hubbard & Cohen 1989a,b, Dent *et al.* 1990a,b).

An essential role of G_M in exercise regulation of GS was recently indicated by a study where G_M was knocked out in mice. After either exercise or *in situ* muscle contraction GS activity was increased in the wild type but unchanged in the G_M knockout mice (Aschenbach *et al.* 2001). This strongly suggests that G_M -associated PP1 activity is essential for the activation of GS after exercise. Based on estimations of the cellular concentrations of G_M and its substrates, Hubbard & Cohen (1989b) reasoned that the relatively large size of the glycogen particles might be expected to introduce constraints to reactions among glycogen-bound proteins. It could be further speculated that high glycogen levels would make it more difficult for the PP1- G_M complex to dephosphorylate GS as glycogen content increases, but as the glycogen content progressively decreases during exercise PP1- G_M would be exposed to interaction with GS, increasing the activity of the enzyme. However, as each G_M subunit binds only one GS protein (Stralfors *et al.* 1985, Hubbard & Cohen 1989c) and it can be estimated that cellular GS protein is in about an eightfold molar excess of the G_M subunits (P. Cohen, personal communication) it is likely that additional or other mechanisms are underlying the potent inhibitory effect of glycogen on GS activity.

PKA

As mentioned above, GS activity is not always increased and in some studies even decreased in response to

exercise. Based on *in vitro* observations several kinases could in theory be responsible for this exercise-induced deactivation of GS (reviewed in Roach 2002). Furthermore, a decrease in phosphatase activity during exercise would also be able to decrease the activity of GS, as phosphate turnover is relatively rapid in GS (Smith & Lawrence 1985). Importantly, an inherent response to exercise is an increase in plasma adrenaline. Adrenaline infusion in resting humans induces a significant drop in skeletal muscle GS activity (Chasiotis *et al.* 1983b, Chasiotis & Hultman 1985). This is thought to be due to an adrenaline-induced increase in cAMP in turn activating PKA, which has been shown to phosphorylate and deactivate GS (reviewed by Cohen 1978). Thus, it could be hypothesized that the exercise-induced drop in GS activity that is sometimes observed, is due to an increase in plasma adrenaline and not due to muscle contraction *per se*. In support of such a view, GS activity has so far not been reported to decrease when rodent muscle is stimulated to contract in the isolated perfused rat hindlimb or when incubated *in vitro* (Adolfsson 1973, Richter *et al.* 1982, Wojtaszewski *et al.* 1999b, Sakamoto *et al.* 2002) – experimental models that eliminates a possible deactivating effect of blood-borne substances on GS (e.g. adrenaline-induced GS deactivation). On the other hand, GS activity has been observed to decrease in rodent skeletal muscle in response to short-term swimming exercise where effects of humoral factors are kept intact (Brau *et al.* 1997). Most importantly, β -adrenergic blockade (propranolol infusion) abolished the decrease in GS activity induced by short-term isometric or dynamic exercise (Chasiotis *et al.* 1983a). Taken together, these observations suggest that the increase in plasma adrenaline during exercise is able to suppress GS activity and that the increase in PKA activity seen during exercise (Yan *et al.* 1992) is due to an increase in adrenaline and/or other β -adrenergic blood borne stimulators and not due to muscle contraction *per se* as cAMP levels are not changed during muscle contraction *in vitro* (Posner *et al.* 1965, Richter *et al.* 1982).

Although site 2 but not site 3 on GS is phosphorylated by PKA *in vitro* (reviewed by Roach 2002) phosphorylation of site 3 is increased upon *in vivo* adrenaline infusion (Parker *et al.* 1982, Poulter *et al.* 1988, Nakielny *et al.* 1991). This indicates that PKA may exert its effect on GS by direct phosphorylation of site 2 but also by indirect mechanisms affecting phosphophorylation of site 3. As mentioned in the previous section, the PP1-subunit G_M is suggested to be a pivotal factor in exercise-induced GS activation (Aschenbach *et al.* 2001). The observation that adrenaline induces phosphorylation of glycogen-bound and SR-bound G_M at Ser48 and Ser67 when injected intravenously into rabbits and rats (Dent *et al.* 1990a, Walker *et al.*

2000) adds further complexity to the exercise regulation of G_M . Phosphorylation of G_M causes release of PP1 from G_M *in vivo* (Hiraga & Cohen 1986, MacKintosh *et al.* 1988) and *in vitro* (Hiraga & Cohen 1986, Hubbard & Cohen 1989a). Taken together with the observations that the activity of released cytosolic PP1 is approximately fivefold lower than the activity of G_M bound PP1 and that PP1 and GS both must bind to G_M in order for G_M to mediate activation of GS (Liu & Brautigam 2000) this suggests an indirect mechanism whereby exercise via adrenaline is able to inhibit GS activity. However, recently Kim & DePaoli-Roach (2002) showed that adrenaline-induced deactivation of GS is intact in G_M knockout mice. This study suggests that although PKA is not thought to act on site 3 of GS other adrenaline-activated kinases may. Alternatively or additionally, another phosphatase/phosphatase complex than the G_M -PP1 complex is involved. Taken together, adrenaline-mediated inhibition of GS is likely to be a major mechanism by which GS activity is inhibited during exercise although the mechanism lying behind the adrenaline effect on GS activity is not fully elucidated.

AMPK

Based on the view that the AMPK enzyme is a sensor of cellular energy charge, turning off ATP consuming anabolic pathways (Hardie & Carling 1997), it could be proposed that AMPK activation may work to decrease the activity of GS during exercise, slowing down the energy-consuming incorporation of UDP-glucose into glycogen. This is supported by the observation that ser7 on GS is a substrate of AMPK *in vitro* (Carling & Hardie 1989) and *in vivo* as detected by a phospho-specific antibody (D.G. Hardie *et al.*, personal communication). Phosphorylation of GS on ser7 increases the susceptibility of ser10 on GS for phosphorylation by CK 1 (Flotow & Roach 1989, Flotow *et al.* 1990, Nakielny *et al.* 1991), and phosphorylation of these two sites on GS decreases the enzyme activity significantly (Flotow & Roach 1989, Nakielny *et al.* 1991, Zhang *et al.* 1993). Furthermore, GS co-immunoprecipitates with AMPK in skeletal muscle (Chen *et al.* 1999). It has been observed that AMPK activation by AICAR decreased GS activity and increased phosphorylation status of GS in skeletal muscle from the perfused rat hindlimb (Wojtaszewski *et al.* 2002) and in rat epitrochlearis muscle incubated *in vitro* (Miyamoto *et al.* 2001). In contrast, a third study reports an unchanged GS activity in rat FDB and epitrochlearis muscle in response to AICAR incubation *in vitro* (Aschenbach *et al.* 2002). In the same study, the effect on GS activity of an intraperitoneal AICAR injection was followed over a 2-h time course. GS activity decreased and increased in

white and red gastrocnemius, respectively, indicating that *in vivo* the AICAR effect is muscle fibre-type specific as also observed in the perfused rat hindlimb (Wojtaszewski *et al.* 2002).

A high muscle glycogen level inhibits AICAR-induced α_2 AMPK activity (Wojtaszewski *et al.* 2002). This coincides with a smaller decrease in GS activity in high glycogen muscle than in low glycogen muscle when perfused with AICAR (Wojtaszewski *et al.* 2002), which is consistent with the view that AMPK activation decreases GS activity. Notably, high muscle glycogen levels are associated with both low AMPK and GS activity (Nielsen *et al.* 2002a, Wojtaszewski *et al.* 2002) strongly suggesting that factors other than AMPK are responsible for the tight relationship between GS activity and glycogen level. In conclusion, AMPK might work as a GS deactivator during exercise but it should be remembered that most studies of the effect of AMPK on GS has applied AICAR and not exercise or muscle contraction as a stimulator of AMPK. AICAR and exercise diverge on the important point that AICAR in contrast to exercise does not lead to glycogen breakdown.

GSK3

GSK3 consecutively phosphorylates site 4, 3a, 3b and 3c on GS when site 5 is phosphorylated by CK 2 (Picton *et al.* 1982, DePaoli-Roach *et al.* 1983, Fiol *et al.* 1987, 1990, Zhang *et al.* 1993). In rodent skeletal muscle, GSK3 activity of the α and β isoforms of GSK3 is decreased in response to treadmill exercise, *in situ* contraction and in isolated muscle contracted *in vitro* in the absence of systemic factors (Markuns *et al.* 1999, Sakamoto *et al.* 2002). These observations coincide with increased phosphorylation of GSK3 β on ser⁹ but not always ser²¹ on GSK3 α (Markuns *et al.* 1999, Sakamoto *et al.* 2002). Thus, GSK3 could be a potential regulator of GS activity during exercise, but while insulin deactivation of GSK3 α is present in human skeletal muscle (Nikoulina *et al.* 2000, Wojtaszewski *et al.* 2000, 2001, Nielsen *et al.* 2002b), the lack of an exercise-induced decrease in GSK3 α and GSK3 β activity (Wojtaszewski *et al.* 2001, Nielsen *et al.* 2002a) questions whether the role of GSK3 as a GS regulator is the same in rodent and human muscle and whether GSK3 has a role in exercise regulation of GS in human skeletal muscle.

G6P

As mentioned in the introductory section, G6P is a direct and indirect stimulator of the activity of GS, although the direct allosteric effect of G6P is not picked up when measuring GS activity *in vitro*. Nevertheless, it should be considered that the G6P may play an important role in regulating GS during exercise. During

exercise the glucose uptake is increased in skeletal muscle and this often leads to increases in the concentration of G6P levels (Richter 1996). Thus, the allosteric activation of GS during exercise could be quite significant as suggested by Piras & Staneloni (1969) and Bloch *et al.* (1994). GS activity is also increased in contracting skeletal muscles stimulated to contract *in vitro* or *in situ* perfused or incubated in the absence of glucose keeping G6P levels very low and unchanged (unpublished observation by W. Derave). This suggests that the increase in GS activity induced by exercise is not dependent on an increase in skeletal muscle G6P levels, which is also supported by observations in human muscle cell cultures (Montell *et al.* 1999).

Summary and conclusion

Taken together, exercise regulation of GS is characterized by great complexity. GS is a substrate of kinases and phosphatases acting on several phosphorylation sites of GS. Furthermore, changes in cellular G6P levels may affect GS by direct allosteric as well as indirect mechanisms. As summarized in Figure 3, exercise activates both stimulatory and inhibitory regulators of GS, with the latter group being intracellularly as well as systemically activated. The mechanisms responsible for inhibition and especially activation are poorly understood. It may be proposed that the resultant activity of GS during exercise may depend on the relative strength of opposing signals. Glycogen breakdown may be considered the major stimulatory signal, possibly involving the G_M -PP1 complex as an essential component, while plasma adrenaline via PKA and at least one other signal independent of blood borne stimuli are proposed to be responsible for the exercise-induced inhibitory signal to GS activity. The latter signal could be mediated by a direct phosphorylation of GS and in this respect it could prove to be important that activation of AMPK (as seen during exercise) leads to phosphorylation of GS and a concomitant drop in GS activity.

From a teleological point of view, one might expect that during contraction all available glucose and ATP is directed towards fuelling the contractions instead of being utilized as substrates for glycogen storage. Therefore, it could seem as a paradox that exercise increases the activity of GS. However, it must be remembered that although it has been demonstrated that glycogen synthesis is increased by exercise (Constable *et al.* 1984, Kuipers *et al.* 1987, 1989, Hutber & Bonen 1989, Gorski *et al.* 1991, Price *et al.* 1991, 1994, Azevedo *et al.* 1998, Huang 1998) exercise almost always results in net glycogen breakdown, most likely reflecting the higher cellular enzymatic potential for glycogen breakdown than glycogen synthesis (Richter *et al.* 1982, Connett & Sahlin 1996). The

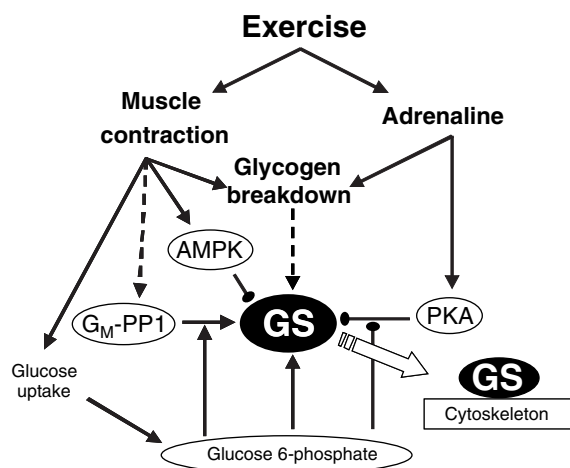


Figure 3 Regulation of skeletal muscle glycogen synthase (GS) during exercise. Exercise elicits both muscle contraction and an increase in blood adrenaline levels, leading to increases in glycogen breakdown and glucose uptake. An exercise-induced decrease in glycogen content stimulates GS activity as the activity of GS is inversely related to glycogen. The mechanism linking glycogen levels and GS activity is not completely understood, but it could prove to be an important clue that GS associates with the cytoskeleton when glycogen levels are decreased. Furthermore, the glycogen-associated PP1 subunit G_M , has been shown to be essential in exercise activation of GS. Adrenaline activates cAMP-dependent protein kinase/protein kinase A (PKA), which decreases GS activity by directly phosphorylating site 2 and indirectly site 3 by a yet unknown mechanism. Glucose 6-phosphate often increases during exercise due to an increase in glucose uptake and an increase in glycogen breakdown. This allosterically activates GS, but glucose 6-phosphate also increases and decreases the susceptibility of GS to the action of GS phosphatases (primarily PP1) and PKA, respectively. Activating and inhibiting factors are indicated by sharp arrowheads and black ovals, respectively. Dotted arrow lines indicate effects that are not completely understood.

exercise-induced increase in GS activity may be part of a functional mechanism of ‘glycogen sparing’ in the exercising muscle, protecting it against complete glycogen depletion during intense exercise or early glycogen depletion during prolonged exercise. Shulman & Rothman (2001) have proposed an intriguing hypothesis that a ‘glycogen shunt’ operates in exercising muscle. According to this hypothesis, glycogen is resynthesized from blood glucose in the period between each muscle contraction, ensuring that energy from glycogenolysis can be rapidly supplied during the following muscle contraction.

Perspectives

Insulin resistance-related diseases are a rapidly growing problem and the search for ways to treat these diseases has therefore been intensified. Pharmaceutical drugs or

eventually gene therapies that mimic the effects of exercise on carbohydrate metabolism and gene expression could prove to be potent therapeutic tools in the treatment of type 2 diabetes and other insulin resistance-related diseases. In order to achieve this, it is essential that the cellular mechanisms and signaling molecules whereby exercise augments glucose uptake and glycogen synthesis are identified. Thus, elucidation of the mechanisms by which exercise regulates GS activity could have major therapeutic perspectives. Future research should be focused on elucidating the specific phosphorylation sites on GS that are affected by exercise and muscle contraction *per se*. This could lead to a better understanding of how exercise regulates GS and which upstream enzymes that are important in this process. Furthermore, it should be investigated whether translocation of GS induced by decreases in glycogen levels renders specific subcellular pools of GS more susceptible to stimulation by insulin or other stimuli.

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