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Novel Aspects of Skeletal Muscle Glycogen and Its Regulation During Rest and Exercise

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SHEARER, J., and T. E. GRAHAM. Novel aspects of skeletal muscle glycogen and its regulation during rest and exercise. *Exerc. Sport Sci. Rev.*, Vol. 32, No. 3, pp. 120–126, 2004. *Although it is often viewed as a homogenous substrate, glycogen is comprised of individual granules or 'glycosomes' that vary in their composition, subcellular localization, and metabolism. These differences result in additional levels of regulation allowing granules to be regulated individually or regionally within the cell during both rest and exercise.* **Keywords:** carbohydrate, glycosome, proglycogen, macroglycogen, glycogenolysis, exercise, glycogen synthase, glycogen phosphorylase

INTRODUCTION

Muscle glycogen is a key modulator of exercise duration, performance, and the onset of fatigue. As such, understanding glycogen metabolism and its regulation was the first topic addressed by Hultman and Bergstrom when they introduced the needle biopsy technique to exercise physiology. Although the basic pathways involved in glycogen synthesis and degradation have been well established, areas that are only now being addressed include (1) protein–protein interactions in glycogen regulation, (2) processes involved in glycogen granule formation and degradation, and (3) the nature of subcellular metabolic compartments. In addition to understanding these processes, an abundance of information has emerged on the role of glycogen in regulating other genes and proteins in skeletal muscle metabolism. Glycogen concentration itself is a potent regulator of insulin sensitivity as well as the activity and expression of GLUT-4, hexokinase, glycogen synthase (GS), glycogen phosphorylase (GP), and pyruvate dehydrogenase kinase 4 (9,10). Although glycogen data often are interpreted as if it is homogenous, close examination of glycogen granules reveals that each is an organelle-type structure containing carbohydrate as well as its own metabolic machinery. This compartmentalized structure allows granules to be potentially regulated both individually and regionally within the cell. It is this heterogeneity of glycogen and the differential regulation of glycogen granules that is the focus of the present review.

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WHAT IS A GRANULE?

A granule is a single molecule of glycogen and consists of chains of carbohydrate and a complement of proteins. Collectively, the entire structure is also known as a *glycosome* (discussed below). A granule begins when uridine diphosphate glucose (UDP)-glucose is directed toward glycogenin, an autoglycosylating protein that initiates glycogen granule formation (12). Glycogenin is both a substrate and an enzyme that catalyzes the addition of 7 to 11 glucosyl units to a specific tyrosine residue on the protein. Once this has occurred, the glucoslyated glycogenin then acts as a substrate for GS and branching enzyme to catalyze the addition of more glucose residues. As more glucose is sequentially added by these two enzymes, the glycogen granule grows in tiers (Fig. 1) around the glycogenin core in a highly ordered manner (8,12). Because one branch (B branches) always gives rise to two branches (A branches) in the subsequent tier, carbohydrate storage increases exponentially (Table 1). The maximal size has been predicted to be 12 tiers (42 nm) as at this point, the high density of glucose on the outer tier results in steric hindrance, precluding the addition of more glucose by GS (8). Given that each tier is 3.8 nm and the outermost tier represents 34.6% of the carbohydrate of the molecule, certain characteristics of the granule can be predicted (Table 1). From tier 3 to 12, the diameter increases 5.4

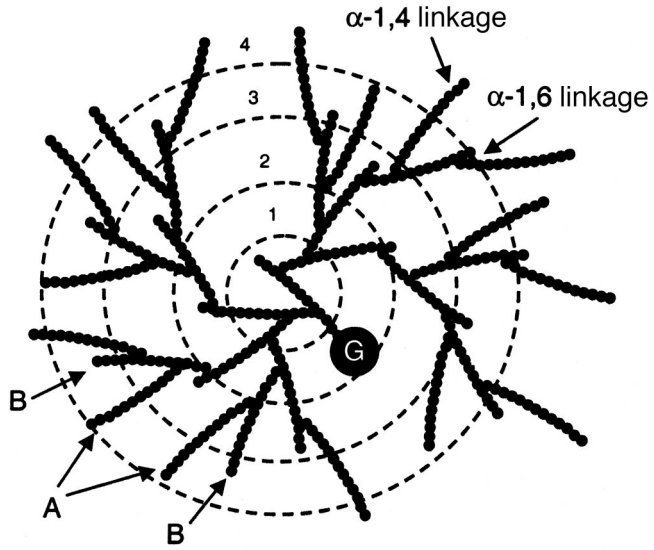

Figure 1. Diagram illustrating the first four tiers of the glycogen granule. Each B chain is linked by α-1–6 glycosidic bonds, whereas each A chain is unbranched and is linked by α-1–4 glycosidic bonds. G, glycogenin, the self-glycosylating protein primer of glycogen. [Adapted from Melendez, R., E. Melendez-Hevia, and M. Cascante. How did glycogen structure evolve to satisfy the requirement for rapid mobilization of glucose? A problem of physical constraints in structure building. *J. Mol. Evol.* 45:446–455, 1997. Copyright © 1997 Springer-Verlag. Used with permission.]

times, but the volume (assuming a sphere) increases 156 times, allowing the carbohydrate content to increase 45.6-fold. The number of A branches also increases exponentially, resulting in progressively more nonreducing ends for GP, the enzyme that catalyzes glycogen catabolism. Although this would suggest that the larger the granule, the more dynamic the glucose turnover, this may not always be the case (see below). This may be because the outer chains are too dense for easy movement of the enzymes or because the huge volume shift inhibits protein–protein interactions.

Recently, we developed a semiquantitative method for assessing glycogen granules (7). The technique uses transmission electron microscopy to assess the number, distribution, and size of individual glycogen granules in skeletal muscle (Fig. 2). This extremely powerful technique can not only can quantify granule appearance and disappearance, but also their distribution between different muscle fiber types during glycogen synthesis and degradation. Examination of resting skeletal muscle shows that granule size is normally distributed (Fig. 3A). The average size is 25 nm (tier 7), and the largest granules have a diameter of 44 nm. The latter is in remarkable agreement with that predicted by Melendez *et al.* (8), who estimated glycogen granule size based on mathematical modeling of the granule (Table 1).

GLYCOGEN METABOLISM AND THE EXERCISE RESPONSE

The cycle of UDP-glucose incorporation into glycogen and the liberation of glucose-6-phosphate from granules is regulated by GS and GP, respectively. It has been understood for decades that these key enzymes are subject to many levels of regulation. They are acted on by kinases and phosphatases and undergo allosteric regulation. GS is the rate-limiting step in glycogen synthesis and is regulated allosterically by glucose 6-phosphate, UDP-glucose, and adenosine monophosphate (AMP). It also is regulated by phosphorylation-dephosphorylation of up to 10 sites on the protein by a wide range of protein kinases (especially GS kinase 3 and casein-II), but only one phosphatase, protein phosphatase-1 (PP1). GS phosphorylation decreases its activity, whereas dephosphorylation increases activity. Similarly, GP is rate limiting and is regulated by phosphorylation-dephosphorylation, but in a reciprocal manner to that of GS. GP is allosterically regulated by AMP and has only one phosphorylation site. This is acted on by one phosphatase (PP1) and one kinase (GP kinase), which in turn is activated by calcium and cAMP protein kinase. GP activity increases with exercise intensity and is more active in the presence of elevated glycogen concentrations (4,14). Decreased GP activity in the presence of low glycogen concentrations is likely a mechanism that conserves glycogen stores and promotes the use of alternative substrates during exercise. It also demonstrates the heterogenic nature of glycogen metabolism. As discussed above, glycogen is found in discrete granules, and key enzymes, such as GS and GP, are among the proteins physically associated with the granule. Changes in granule volume result in changes in carbohydrate availability, the protein-to-carbohydrate ratio, and the surface-to-volume ratio. These factors influence the interaction of GS and GP with the outer branches of the granule and thus may account for the varied activities of these enzymes with glycogen concentration.

THE GLYCOSOME

As previously mentioned, glycogen granules not only contain carbohydrate, but also proteins involved in their metabolism. Because they contain their own metabolic machinery, granules have been referred to as *organelle-type structures*, or

TABLE 1
Theoretical number of glucosyl residues in each glycogen tier and their predicted diameter

Tier No.	Diameter (nm)	Glucosyl Units per Tier	Total No. of Glucosyl Units
3	7.8	417	1205
4	11.6	637	1842
5	15.4	974	2816
6	19.2	1489	4305
7	23.0	2277	6582
8	26.8	3481	10063
9	30.6	5323	15386
10	34.4	8139	23525
11	38.2	12445	35970
12	42.0	19030	55000

[Adapted from Melendez, R., E. Melendez-Hevia, and M. Cascante. How did glycogen structure evolve to satisfy the requirement for rapid mobilization of glucose? A problem of physical constraints in structure building. *J. Mol. Evol.* 45:446–455, 1997. Copyright © 1997 Springer-Verlag. Used with permission.]

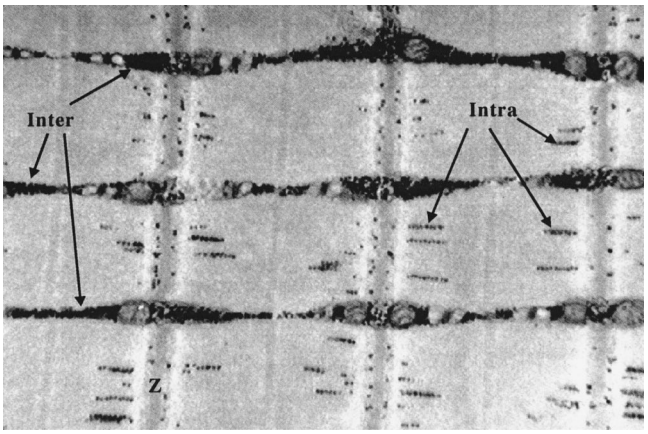

Figure 2. Human skeletal muscle image of muscle glycogen granules. The image was obtained using transmission electron microscopy (original magnification, ×20,000). Intermyofibril glycogen is defined as glycogen located between the myofibrils, whereas intramyofibril glycogen is located within myofibrils. (Reprinted from Marchand I., K. Chorneyko, M. Tarnopolsky, S. Hamilton, J. Shearer, J. Potvin, and T.E. Graham. Quantification of subcellular glycogen in resting human muscle: granule size, number, and location. *J. Appl. Physiol.* 93:1598–1607, 2002. Copyright © 2002 The American Physiological Society. Used with permission.)

glycosomes (Fig. 4). Under normal conditions, each individual granule is associated with a full complement of enzymes that represents 66–80% of its total weight. These associations appear to be very dynamic as individual proteins associate or dissociate, depending on cellular conditions. Proteins in the glycosomes include GS, GP, kinases, phosphatases, glycogenin, branching enzyme, debranching enzyme, and cytoskeletal actin. More recently, additional proteins have been added to this list. Although it is beyond the scope of this review to give a detailed overview all of these additions (see Roach (12)), a brief description of these proteins and their role in glycogen regulation is warranted.

Among the proteins recently identified with glycosomes are glycogenin-interacting proteins (15). These small proteins are expressed in skeletal muscle and stimulate glycogenin glycosylation. Although the roles of glycogenin-interacting proteins have yet to be elucidated, they may be involved in the regulation of glycogen granule initiation (12). Specifically, they may regulate the ability of glycogenin to glycosylate, thus imposing an additional site of regulation. Another emerging protein that seems both to regulate, and be regulated by, glycogen is AMP kinase (AMPK). AMPK is a protein activated in response to alterations in the energy charge of the cell, including exercise, hypoxia, and starvation. Activation of AMPK results in increased glucose and fatty acid uptake, enhanced substrate oxidation, and a decline in substrate storage. In skeletal muscle, AMPK activity is dependent on glycogen concentration. Increases in glycogen concentration lead to declines in AMPK activity, whereas declines in glycogen concentration have the opposite effect (5). Recent studies indicate that AMPK has a glycogen-binding domain and that it colocalizes with both GS and GP (11). Although the exact relationship between AMPK and glycogen is incompletely understood, there is little doubt that they are codependent and reciprocally regulated.

Insights into existing proteins in the glycogen granule have also led to exciting findings on glycogen granule regulation. Of specific interest is PP1, the phosphatase responsible for the majority of GS and GP phosphorylation. Although this protein has been identified for some time, the intricacies of its regulation and metabolic role in glycogen metabolism are just beginning to emerge. The activity of PP1 is mediated in part by the binding of regulatory and accessory subunits. In skeletal muscle, these regulatory subunits are G_M/R_{GL}, protein targeting to glycogen (PPP1R5/PTG), and PPP1R6. An accessory protein, laforin, is also known to colocalize with PPP1R5/PTG, although its role in glycogen metabolism has yet to be elucidated (3,12). Collectively, they organize the glycogen granule, localizing and positioning enzymes in relation to each other and to the outer branches of the granule. G_M/R_{GL} is of particular interest because it contains a sarcoplasmic binding domain, suggesting that it is responsible for anchoring glycogen to specific subcellular

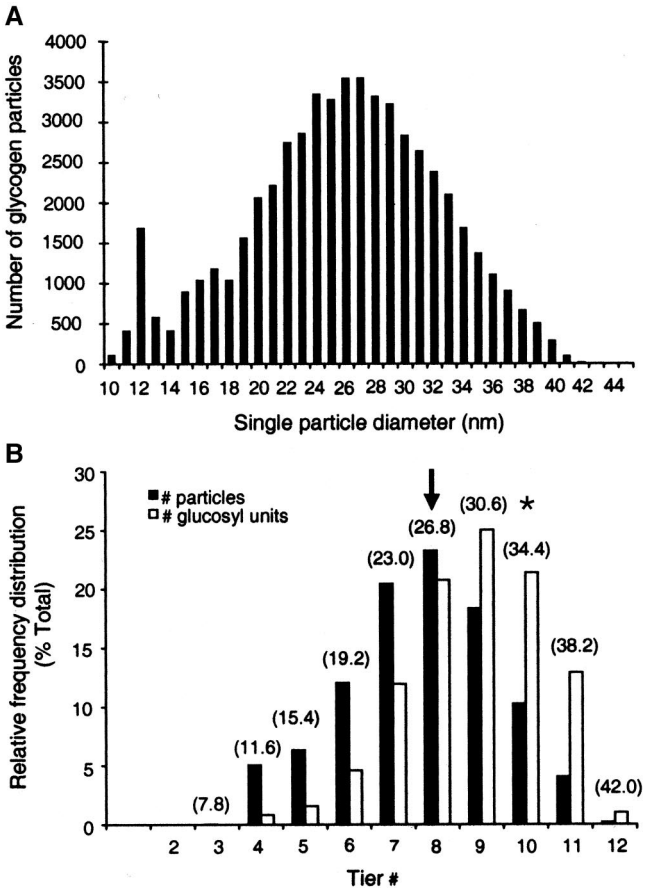

Figure 3. A. Frequency distribution of single glycogen granule diameters. Data represent diameter (nm) frequency from 11 subjects and more than 55,000 glycogen granules. B. Relationship between number of glucosyl units in glycogen granules of various sizes. The values in brackets over the histogram bars represent the actual particle diameters, that is, they correspond with the x-axis of Figure 3A. The black arrow at tier 8 represents the PG-to-MG transition as predicted by Melendez *et al.* (8), whereas the asterisk at tier 10 represents the PG-to-MG transition predicted by Marchand *et al.* (7). (Reprinted from Marchand I., K. Chorneyko, M. Tarnopolsky, S. Hamilton, J. Shearer, J. Potvin, and T.E. Graham. Quantification of subcellular glycogen in resting human muscle: granule size, number, and location. *J. Appl. Physiol.* 93:1598–1607, 2002. Copyright © 2002 The American Physiological Society. Used with permission.)

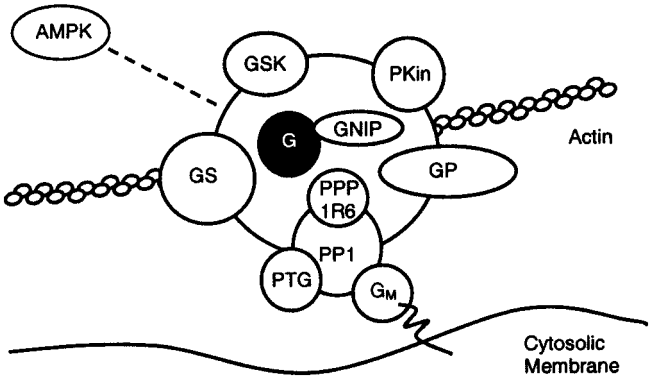

Figure 4. Diagram of a typical glycogen granule. Glycogen granules are known to be associated with cytoskeletal actin that facilitate its movement. GS, glycogen synthase; GP, glycogen phosphorylase; PTG, protein targeting to glycogen; PP1, protein phosphatase 1; G, glycogenin; Pkin, phosphorylase kinase; GSK, glycogen synthase kinase; GNIP, glycogenin interacting protein; G_M/PPPIR6, subunits of PP1; AMPK, adenosine monophosphate kinase. (Reprinted from Shearer, J., and T.E. Graham. New perspectives on the storage and organization of muscle glycogen. *Can. J. Appl. Physiol.* 27:179–203, 2002. Copyright © 2002. Used with permission.)

locales. Presumably, the huge changes in the volume of the granule during metabolism would present great challenges to the protein–protein interactions. Such a regulatory role could explain patterns of glycogen distribution in skeletal muscle and why certain depots of glycogen are metabolized over others (see below).

ALL GLYCOGEN GRANULES ARE NOT EQUAL

Glycogen granules appear to have similar protein contents regardless of size. Thus, those that are small contain a proportionally higher percentage of protein. This results in them being insoluble in acid extraction, and at one time, they were referred to as being acid insoluble. More recently, the term *proglycogen* (PG) has been introduced to reflect that they were smaller molecules that could "grow" larger and as they gained carbohydrate (5). As more carbohydrate is added, the acid solubility of the granules also increases and they are termed *macroglycogen* (MG). The PG granules have been proposed to range in size from glycogenin to 400,000 Da (8 tiers), whereas MG would range from 400,000 Da to 10^7 Da (tier 12).

Originally, Lomako *et al.* (6) described PG and MG as two separate pools of glycogen. Specifically, PG was a 400,000-kDa intermediate between glycogenin and MG. This PG intermediate was thought to accumulate in skeletal muscle, to have its own form of GS, and to be less metabolically active. However, this has not been confirmed in subsequent investigations. Regardless, this theory led Melendez *et al.* (8) to hypothesize that tier 8 would be the largest of PG. Although these are important concepts, when one considers additional evidence, it seems unlikely that they are valid. For example, subsequent investigations have failed to confirm that PG and MG are distinctly separate granule sizes, but rather are found in a continuum in sizes. Transmission electron microscopy analysis of granules by Marchand *et al.* (5) clearly substantiate this in human muscle (Fig. 3A). Although there is considerable evidence that PG and MG are not separate pools, acid solubility is a useful way to divide smaller and larger granules. This has been found to provide a deeper understanding of glycogen metabolism in that the two fractions do not respond in the same manner to metabolic perturbations (4,14). In addition, when we incorporate our data on granules with that of the model outlined by Melendez *et al.* (8) for the carbohydrate content of each tier, further understanding is obtained. Because of the exponential relationship of granule size and carbohydrate content, the distribution of carbohydrate by granule size does not follow the same normal distribution. In Figure 3B, one can see that although size is normally distributed, carbohydrate content is not; for example, tier 7 has more than 20% of the granules but 12% of the carbohydrate, whereas tier 10 has 10% of the granules and 22% of the carbohydrate, and tier 11 has only 4% of the granules and 13% of the carbohydrate. In addition, based on the total glycogen content (380 ± 41 mmol·kg^{-1}dw), the proportion that was PG (74%), and the estimate for the carbohydrate content of granules of various tier sizes (Table 1), we can predict the distribution of carbohydrate by granule size and where the transition from PG to MG occurs. Rather than it being at tier 8, we predict that it occurs at tier 10 (Fig. 3B). The predicted transition at tier 8 (8) would mean that 32–33% of the granules were MG, whereas tier 10 transition means that it is between 4–14%. This has important implications in the interpretation of the PG and MG data.

Over several decades, some scientists have determined muscle glycogen based on acid solubility (PG and MG). When the data are examined in this fashion, it becomes apparent that PG and MG have different metabolic behaviors. During recovery from prolonged exercise, human skeletal muscle initially produces predominantly PG. When it

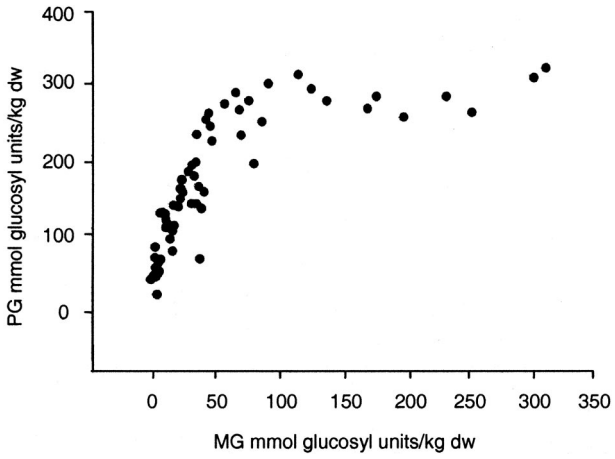

Figure 5. Distribution of PG and MG in skeletal muscle with increasing glycogen concentration. Total glycogen is the sum of PG plus MG. For example, at MG concentrations of 100, 200, and 300 mmol·kg^{-1}dw, the PG is approximately 250 mmol·kg^{-1}dw and total glycogen is approximately 350, 450, and 550 mmol·kg^{-1}dw. (Reprinted from Adamo, K.B., M.A. Tarnopolsky, and T.E. Graham. Dietary carbohydrate and postexercise synthesis of proglycogen and macroglycogen in human skeletal muscle. *Am. J. Physiol.* 275:E229–E234, 1998. Copyright © The American Physiological Society. Used with permission.)

achieves a glycogen concentration of 300 to 400 mmol·kg^{-1}dw, PG is approximately 75% of the total glycogen. As glycogen concentration increases above this level (supercompensation), additional carbohydrate is added as MG, but there is no corresponding decline in PG (Fig. 5). From these data, it is not clear whether the increase in MG is the result of the addition of glucose to all existing granules, with some then becoming acid soluble (MG), or whether there is an expansion in granule number as well. In our laboratory, it was found that glycogenin was directly related to glycogen storage, suggesting that expansion of stores is at least in part the result of an increase in granule number (13). Elsner *et al.* (2) recently reported findings that support this possibility; using a myotube cell culture and labeled glucose, they were able to show that when the cells synthesized glycogen, there was an increase in both granule size and number. These various observations consistently imply that there is a preference to more moderate-sized granules rather than fewer large ones.

Under many exercise conditions, PG is more dynamic. At low to moderate exercise intensities (>70% $\dot{V}O_2$max), an equal proportion of glucose is derived from the PG and MG pools. However, as exercise intensity increases, glucose is predominantly derived from PG (14) (Fig. 6). The dependence on PG could be due to the low proportion of MG granules; however, when exercise is repeated, MG catabolism can be dramatically suppressed (4). Earlier investigations that used acid solubility to determine glycogen concentration also showed that MG catabolism was markedly inhibited during repeated sprints (4). Therefore, it seems that PG granules are more metabolically favored compared with MG (13). Consistent with these observations, Elsner *et al.* (2) found that when glycogen was catabolized in myotubes, degradation of granules was largely sequential or out of phase, with some granules being catabolized, whereas others remained untouched. This variable metabolism between granules as well as PG and MG may be teleological. PG seems to be a readily expendable source of glucose, whereas MG acts as a reserve only used under extreme conditions, such as low glycogen stores or prolonged, high intensity exercise. Such a metabolic distinction also would explain why not all PG is converted to MG, even at the highest observable glycogen concentrations (Fig. 5). Differential regulation between PG and MG may stem from variations in the way GS and GP interact with glucose residues on the outer tiers of the granule. As previously discussed, these metabolic differences may be the result of physical differences in glycogen–enzyme interactions. PG, being a smaller molecule, would have less steric hindrance and possibly more favorable interactions with GS compared with MG. The low proportion of granules that are MG may also contribute to these differences.

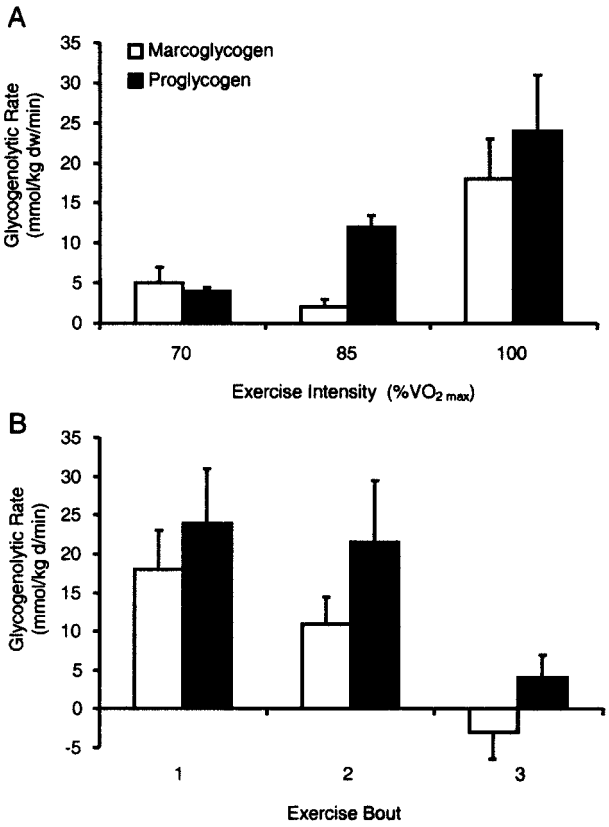

Figure 6. A. Rates of glycogenolysis in PG and MG and total glycogen (G_t = PG + MG) at 70, 85, and 100% $\dot{V}O_2$max during cycle ergometry. B. Rates of glycogenolysis during repeated cycle ergometry exercise (3 × 3 min at 100% $\dot{V}O_2$max). Data represent mean ± SEM and were obtained from human muscle biopsies. Different letters above bars indicate significant differences ($P < 0.05$). See (4) for study details. (Reprinted from Shearer, J., and T.E. Graham. New perspectives on the storage and organization of muscle glycogen. *Can. J. Appl. Physiol.* 27:179–203, 2002. Copyright © 2002. Used with permission.)

GRANULES IN MOTION

Physically, granules are not randomly distributed, but rather highly organized into specific subcellular locales. On visualization by transmission electron microscopy, glycogen granules are primarily distributed in three main depots: the intermyofibrillar space, the intramyofibrillar space, and in the subsarcolemmal space (7). Within each of these locations, granules are not uniform, but are organized further into compartments. In the intermyofibrillar space, granules are clustered near mitochondria, close to the Z disc and the t-tubules. In comparison, granules in the intramyofibrillar space are highly organized, extending out from the Z disc in straight lines, apparently in association with actin (Fig. 5). It has been suggested that the location of granules dictates function. There have been proposals that the very densely packed and smaller granules of the subsarcolemmal space supply mitochondria that are dedicated to H^+ ion pumps, and those near the sarcoplasmic reticulum are similarly serving Ca^{2+}ATPase pumps. These theories remain to be addressed in detail.

Although it is not known if granules can move, at least some of the machinery involved in glycogen metabolism is dynamic. Nielsen *et al.* (9) demonstrated this using GS. Using differential centrifugation, confocal microscopy, and protein blotting, they showed that GS (normally associated with glycogen granules) translocates from glycogen-enriched outer membranes to the inner cytoskeleton when glycogen levels are depleted. The authors hypothesized that this may occur to supply energy nearby the contractile apparatus. In

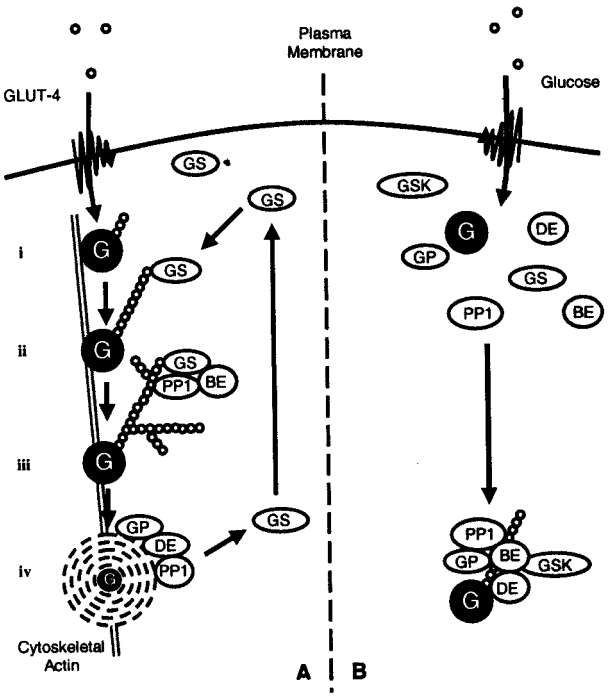

Figure 7. Proposed scheme of glycogen granule formation in skeletal muscle. A. (*i*) Glycogenin initiates glycogen granule formation at the sarcolemmal membrane; (*ii*) once initiation is complete, glycogen synthase attaches to the granule, resulting in its growth; (*iii*) as soon as granules are of sufficient size, other glycogen-metabolizing enzymes attach to the granule; (*iv*) glycogen synthase dissociates from the mature granule and returns to the sarcolemmal membrane, whereas the granule is translocated to its final location on actin filaments. B. Incoming glucose is transported to the site of the granule. Here, initiation and the association of other proteins in the glycosome occur at the site of final granule location. G, glycogenin; GP, glycogen phosphorylase; GS, glycogen synthase; BE, branching enzyme; DE, debranching enzyme; PP1, protein phosphatase 1.

addition, this study demonstrated that glycogen content itself is a more potent determinant of GS activity and physical localization of this enzyme compared with insulin (9).

Another unknown is how and where glycogen granules are assembled. Does all assembly occur at the final location of the granule or does it begin near the site of glucose entry and then position itself within the cell as metabolism demands (Fig. 7)? As previously mentioned, GS translocates depending on glycogen concentration. This raises the question of whether other proteins also translocate depending on metabolic demands. Current evidence supports this hypothesis. Glycogenin contains a binding domain for cytoskeletal actin, suggesting it may track along actin filaments to various subcellular locations (1). If this is the case, then glycogen granule initiation may begin close to the site of glucose entry and then translocate elsewhere in the cell where other proteins bind to the glycosome (Fig. 7).

GLYCOGEN AS A METABOLIC REGULATOR

Understanding glycogen regulation not only has implications for skeletal muscle metabolism, but also whole-body glucose homeostasis. Numerous studies have demonstrated that muscle glycogen concentration itself is a powerful mediator of insulin sensitivity, protein activity, and gene expression. After glycogen depletion, insulin sensitivity is highest 2 h after exercise when carbohydrate is available, but can remain elevated for up to 48 h if glycogen stores are not replenished. During glycogen depletion and the subsequent repletion, numerous proteins and genes (mRNA) are upregulated, presumably to facilitate repletion of glycogen stores. These genes include GLUT-4, hexokinase II, uncoupling protein 3, lipoprotein lipase, interleukin-6, and pyruvate dehydrogenase kinase 4, among others (10). Recently, Pilegaard *et al.* (10) demonstrated that pyruvate dehydrogenase kinase 4, hexokinase II, uncoupling protein 3, and lipoprotein lipase mRNA are upregulated to a greater extent when glycogen concentrations are low. This has led to the hypothesis that glycogen depletion and upregulation of metabolic genes in skeletal muscle are coordinately regulated. Indeed, after glycogen depleting exercise, peak mRNA levels occur 1 to 4 h in recovery. This suggests that there may be a factor associated with the glycosome that is released on glycogen degradation that induces the transcription of metabolic genes.

CONCLUSIONS

In summary, glycogen is a dynamic molecule whose regulation is mediated at various levels during rest and exercise. Although GS and GP are the primary enzymes involved in glycogen metabolism, other factors, such as glycogen granule size, subcellular localization, and interaction with other proteins, contribute to the metabolic regulation of glycogen. Additionally, glycogen is becoming recognized as a mediator of other proteins and enzymes in skeletal muscle. Given this, understanding this molecule is of importance not only to exercise metabolism, but also to whole-body glucose homeostasis.

Acknowledgments

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