Glycogen branches out: new perspectives on the role of glycogen metabolism in the integration of metabolic pathways

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Glycogen branches out: new perspectives on the role of glycogen metabolism in the integration of metabolic pathways. Am J Physiol Endocrinol Metab 291: E1–E8, 2006; doi:10.1152/ajpendo.00652.2005.—Glycogen is the storage form of carbohydrate for virtually every organism from yeast to primates. Most mammalian tissues store glucose as glycogen, with the major depots located in muscle and liver. The French physiologist Claude Bernard first identified a starch-like substance in liver and muscle and coined the term glycogen, or “sugar former,” in the 1850s. During the 150 years since its identification, researchers in the field of glycogen metabolism have made numerous discoveries that are now recognized as significant milestones in biochemistry and cell signaling. Even so, more questions remain, and studies continue to demonstrate the complexity of the regulation of glycogen metabolism. Under classical definitions, the functions of glycogen seem clear: muscle glycogen is degraded to generate ATP during increased energy demand, whereas hepatic glycogen is broken down for release of glucose into the bloodstream to supply other tissues. However, recent findings demonstrate that the roles of glycogen metabolism in energy sensing, integration of metabolic pathways, and coordination of cellular responses to hormonal stimuli are far more complex.

GLYCOGEN STRUCTURE AND KEY ENZYMES OF ITS METABOLISM

GLYCOGEN SYNTHESIS follows a simple but strictly ordered process, resulting in a complex structure (38, 54). The initiation of glycogen synthesis is provided by self-glucosylation of glycogenin (55). To this oligosaccharide primer, glycogen synthase utilizes UDP-glucose to add glucose molecules by α1–4 linkages, which is the rate-controlling step of glycogen synthesis. Glycogen synthase activity is controlled by covalent modification, allosteric activation, and enzymatic translocation. The enzyme is phosphorylated on up to nine residues, resulting in its progressive inactivation and decreased sensitivity to allosteric activators (31). Binding of glucose 6-phosphate (G-6-P) to glycogen synthase causes unfolding of the enzyme, resulting in allosteric activation that overrides inhibition by phosphorylation. In addition, G-6-P binding promotes conformational changes that favor dephosphorylation of the enzyme. Translocation of glycogen synthase to glycogen particles in response to stimuli such as insulin represents a third mechanism by which enzymatic activity is regulated (13, 42, 49). When the elongating glycogen chain consists of at least 11 residues, branching enzyme transfers a chain of seven molecules to another chain by an α1–6 bond. Thus glycogen synthase elongates the glycogen chain, and branching enzyme produces new branches, creating a molecule with a helical structure of 12 concentric tiers (38).

For glycogen degradation, the synchronous activities of glycogen phosphorylase and debranching enzyme are required. Glycogen phosphorylase, which catalyzes the rate-limiting step of glycogenolysis, cleaves α1–4 linkages to remove glucose molecules from the glycogen chain. When four glucose units remain before a branch point, the transferase activity of debranching enzyme catalyzes the transfer of three glucose residues to an adjacent branch of the glycogen chain. Through a second enzymatic glucosidase component located in the same protein, debranching enzyme next cleaves the α1–6 bond to release the glucose moiety from the branch point. Glycogen phosphorylase is then able to continue removal of glucose residues from the glycogen chain (38).

Glycogen phosphorylase is regulated by phosphorylation of a single serine residue at the NH2 terminus and by allosteric binding of a number of molecules, including AMP, ATP, G-6-P, glucose, and caffeine (25). The enzyme exists as a dimer with each subunit linked to the essential cofactor pyridoxal phosphate, which provides the phosphate as an electron donor for release of glucose 1-phosphate. The covalent modification of glycogen phosphorylase in response to changes in intracellular cAMP and calcium levels is mediated by the activation of phosphorylase kinase, the sole enzyme that phosphorylates and activates glycogen phosphorylase (5). Thus both glycogen synthase and phosphorylase are regulated by multiple mechanisms that enable glycogen metabolism to be acutely responsive to the varying metabolic demands in both liver and skeletal muscle cells.

SKELETAL MUSCLE GLYCOGEN

During rest, storage of glucose as glycogen is enhanced by insulin stimulation of glucose uptake in muscle, which is mediated by the translocation of GLUT4 transporters to the plasma membrane (62). Entering glucose is phosphorylated to...
G-6-P by hexokinase II and may enter glycolysis to produce ATP or be converted by phosphoglucomutase to glucose 1-phosphate, which is further metabolized for glycogen synthesis. Additionally, insulin induces the dephosphorylation of glycogen synthase via inhibition of upstream kinases and activation of phosphatases (4). Thus insulin synergistically stimulates glycogen synthesis in muscle by coordinate-ly increasing intracellular concentrations of G-6-P and by dephosphorylation and activation of glycogen synthase.

In humans, skeletal muscle is the primary site for glucose disposal, where up to 90% of a glucose load is converted to glycogen (26). Skeletal muscle fibers differ in the amount of glycogen they store and are classified as type I (fast switch) or type II (slow switch), based on their contractile speed and metabolic properties. Type I fibers are responsible for slow-speed contractions and utilize oxidative pathways for ATP production, whereas type II fibers are capable of rapid contractions and rely primarily on glycolytic pathways for energy production. Type II fibers are further classified as type IIa and type IIb; IIa fibers make use of both oxidative and glycolytic pathways for energy production, whereas IIb rely primarily on glycolysis. Thus type I fibers rely more on free fatty acid (FFA) oxidation during contraction, whereas type II fibers are more dependent on glucose uptake and glycolysis.

Muscle glycogen metabolism can be viewed simply as the major site of insulin-stimulated glucose storage during times of energetic abundance, and conversely, glycogen is broken down to provide ATP to the working muscle during exercise. However, recent work in transgenic animals has challenged the notion that muscle glycogen levels are essential for exercise, as modulation of muscle glycogen stores did not impact on exercise performance (45, 46). Furthermore, because glycogen stores are finite, their depletion results in the switching of energy utilization to extracellular glucose and FFA as exercise continues (21). A number of factors may influence when and to what extent glycogen and other fuels are utilized to maintain ATP homeostasis, including the intensity of the exercise, the dietary supply of carbohydrate or lipid, and the training status of the individual (21). In this section, we consider energy utilization in muscle before, during, and after a single bout of exercise in order to demonstrate that glycogen metabolism is a key regulator that integrates multiple energetic pathways.

**Glycogen Metabolism During Exercise**

**Immediate fuel utilization.** At the beginning of a bout of exercise, contraction of skeletal muscle results in the hydrolysis of ATP and a transient rise in ADP and P_i levels. Creatine phosphate/creatine kinase acts as a cellular buffer for ATP levels by providing a source of P_i that can be added to ADP to form ATP by creatine kinase. However, this system is quickly overwhelmed during sustained exercise, and increasing ADP levels stimulate the activity of adenylate kinase, which catalyzes the conversion of two ADP molecules to one ATP and one AMP molecule. As the ATP from this pathway is rapidly consumed, the AMP-to-ATP ratio rises, initiating a number of metabolic events geared toward maintaining energy required for contraction.

**Regulation of glycolysis by glycogen levels.** As the aforementioned immediate energy sources are depleted, the muscle cell must then rely on the glycolytic pathway to provide ATP for continued contraction. Glycolysis is initially activated by the increases in AMP and P_i, which stimulate the rate-limiting enzyme in glycolysis, phosphofructokinase. Glycolysis is stimulated as well by an increase in provision of G-6-P to this pathway, which occurs at the onset of exercise initially due to augmented rates of glycogenolysis and, as exercise continues, through an increase in glucose uptake via the contraction-induced, insulin-independent translocation of GLUT4 vesicles to the plasma membrane (24). The metabolism of G-6-P by the glycolytic pathway results in the generation of fructose 2,6-bisphosphate, which is a potent allosteric activator of phosphofructokinase, and thus a key metabolic regulator of glycolytic rates and ATP production (53).

A variety of stimuli are integrated in the regulation of glycogen phosphorylase activity, allowing glycogen breakdown to change in parallel with the energy demands of the exercising muscle. The onset of contraction results in the release of calcium stores from the sarcoplasmic reticulum, causing the activation of phosphorylase kinase and subsequent phosphorylation and activation of glycogen phosphorylase. The majority of glycogen phosphorylase and phosphorylase kinase in the cell is found at the sarcoplasmic reticulum bound to glycogen (32, 39, 63), allowing for the coupling of contraction, calcium release, and glycogen breakdown. Glycogen phosphorylase is also allosterically activated by increased [P_i] within the cell, which rises as ATP levels fall, reflecting an energy deficit. As activity continues, glycogen phosphorylase may also be activated in response to increased extracellular epinephrine levels, which result in activation of PKA, which in turn phosphorylates and activates phosphorylase kinase.

Tight metabolic coupling exists between glycogen mobilization and ATP production via glycolysis. In fact, studies indicate that the G-6-P arising from muscle glycogen breakdown is more efficiently coupled to glycolysis than that arising from GLUT4 and hexokinase, particularly during high-intensity exercise, when 80% of the glucose consumed by glycolysis arises from glycogenolysis (56). Additionally, at moderate-intensity exercise, the rate of glycogenolysis greatly exceeded the rate of glucose transport, suggesting a greater contribution of glycogen breakdown to maintenance of ATP levels. Thus, at the onset of exercise, the provision of G-6-P to the glycolytic pathway from glycogen breakdown would inhibit extracellular glucose transport by both allosteric inhibition of hexokinase II and diminishment of the glucose concentration gradient across the plasma membrane.

**Glucose transport during exercise.** Due to the finite nature of glycogen stores, as contraction continues glycogen phosphorylase activity decreases to prevent complete depletion of cellular glycogen levels (7). The release of glycogen phosphorylase from glycogen partially accounts for the observed reversal in glycogenolysis (21). As glycogen breakdown diminishes during continued contraction, the unfavorable conditions for extracellular glucose uptake and utilization are dissipated. Glucose transport into the muscle is increased, correlated acutely with enhanced GLUT4 translocation to the plasma membrane (24, 52), and, in trained individuals, upregulation of GLUT4 mRNA and protein levels (20, 30). Several studies indicate that muscular glycogen content prior to exercise also affects the regulation of glucose transport (2, 12), although others report no effect (1, 18). The differential effects on glucose uptake seen in these studies could be due to differences
in the exercise intensity of the protocols. In the studies where moderate-intensity exercise without fatigue is used, the observed increases in fat oxidation may compensate for the reduction in muscle glycogenolysis in the low-starting-glycogen groups, such that glucose transport into the muscle was not elevated. However, at higher intensities, when glycogen stores are significantly depleted and lipid metabolism becomes limiting, an increase in glucose uptake would become necessary to fuel continued muscular contraction.

**Regulation of fuel partitioning by glycogen metabolism.** In addition to increasing glucose transport as glycogen stores become depleted, skeletal muscle can also oxidize FFA to produce ATP to preserve glycogen levels. Support for the role of glycogen levels in regulating the switch in fuel utilization by muscle during exercise comes from studies where glycogen content was manipulated prior to exercise (1, 2, 9, 18, 65). In each of these studies, increased muscle glycogen storage prior to exercise led to increased carbohydrate oxidation during exercise compared with controls. Conversely, decreasing muscle glycogen stores prior to exercise decreased carbohydrate oxidation and increased lipid oxidation. Studies also found that subjects with low glycogen prior to exercise displayed increased norepinephrine and FFA levels during exercise (65). These findings are similar to observations made in McArdle’s patients, who lack functional muscle glycogen phosphorylase and cannot break down glycogen stores (33). Substantial evidence suggests that the inability to mobilize muscle glycogen in McArdle’s patients can be directly communicated to the central nervous system, in turn promoting lipid utilization (64, 65). Further evidence supporting a role for glycogen in the regulation of substrate utilization during exercise comes from a recently described transgenic mouse model that lacks the muscle isoform of glycogen synthase and thus muscle glycogen stores (44). The authors observed no change in exercise capacity compared with wild-type animals (46), but there was an increase in glycogen synthase activity in the gastrocnemius muscle, indicative of a compensatory enhancement in lipid oxidative capacity. So, although the exercise capacity of the transgenic mice was unaffected by the loss of muscle glycogen stores, the adaptive differentiation of oxidative type I fibers indicates that, physiologically, muscle glycogen stores serve as an important energy source during exercise.

Cumulatively, these observations reveal that the metabolic changes resulting from glycogen depletion during exercise promote the utilization of FFA for ATP generation. Furthermore, repeated or sustained loss of skeletal muscle glycogen, through exercise training or in transgenic animals, results in adaptive responses at the levels of gene transcription and muscle fiber type differentiation to increase lipid oxidative capacity. These adaptive changes in substrate utilization are geared toward the preservation of finite muscle glycogen stores (47).

**Role of AMPK in fuel utilization.** Recent work has suggested that the enzyme AMP-activated protein kinase (AMPK) may be the molecular link between alterations in glycogen levels and the timing of the shift from carbohydrate to lipid metabolism during exercise (66). AMPK is a key regulator of cellular energy metabolism due to its activation by increases in the AMP/ATP ratio (17) and its effects on increasing energy production and inhibition of energy storage, particularly as glycogen. AMPK regulates numerous cellular processes, including increased oxidation of lipids, enhanced glucose transport, and several of the long-term adaptations associated with exercise, such as upregulation of GLUT4 expression and increased mitochondrial content (6, 67). AMPK inactivates glycogen synthase (68), inhibiting glycogen synthesis in an effort to shunt glucose delivery from storage to glycolysis.

Several lines of evidence suggest that changes in intracellular glycogen stores may regulate AMPK activation. First, an inverse relation between high glycogen levels and AMPK activity is logical and has been observed in rat and human skeletal muscle (11, 68, 69). Conversely, low muscular glycogen levels at the onset of exercise enhanced AMPK activation compared with high muscle glycogen controls (11, 68, 69). Finally, the loss of glycogen stores in the muscle glycogen synthase knockout mouse model correlated with a robust increase in AMPK phosphorylation state (44). AMPK has been shown to directly bind glycogen (48), but because glycogen addition did not affect AMPK activity in vitro (48), the significance of this interaction may be at the level of subcellular localization of the kinase rather than a direct modulation of enzymatic activity. Thus, during sustained exercise, as glycogen stores are exhausted, activation of AMPK enables muscle fibers to switch over to energy utilization from extracellular glucose (via increased GLUT4 translocation) and FFA oxidation (via inhibition of acetyl-CoA carboxylase, the rate-limiting enzyme for fatty acid synthesis).

**Glycogen Metabolism During Recovery**

**Regulation of cellular responses to hormonal input.** After a strenuous bout of exercise, skeletal muscle is geared toward increased energy uptake and metabolism to produce ATP and, subsequently, repletion of glycogen stores. Two of the hallmark features of recovery from glycogen-depleting exercise are the increased response and sensitivity to insulin, resulting in enhanced GLUT4 translocation and glucose uptake (29), and activation of glycogen synthase following refeeding. In exercised rats, glucose uptake was significantly greater in muscle with low glycogen compared with high-glycogen muscle when perfused at the same insulin concentration (12). Furthermore, it was found that the rate of glycogen synthesis limited the rate of glucose transport, as opposed to the availability of intracellular substrate, indicating that transport itself was not limiting.

The elevation in glycogen synthase activity after glycogen depletion may be regulated at the level of protein translocation. As is the case with glycogen phosphorylase, glycogen synthase binds directly to glycogen (39); yet basal and insulin-stimulated glycogen synthase activity is negatively correlated to glycogen levels (3, 10, 42). Using a subcellular fractionation technique, Nielsen et al. (42) demonstrated that, in glycogen-depleted muscle, glycogen synthase is found in a cytoskeleton fraction. Furthermore, enzymatic activity was enhanced to a greater extent in the low-glycogen than in the high-glycogen rats in response to both insulin and muscle contraction, suggesting that glycogen stores dictate the subcellular localization of glycogen synthase, which in turn correlated with alterations in modulation of glycogen synthase activity (42). In a recent study, Prats et al. (49) demonstrated the redistribution of glycogen synthase in a phosphorylation-dependent manner to

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AJP-Endocrinol Metab • VOL 291 • JULY 2006 • www.ajpendo.org

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an intracellular compartment where glycogen reassembly occurred following depletion of glycogen stores (49). However, the enzymatic mechanisms underlying alterations in glycogen synthase subcellular distribution and degree of enzymatic activation remain poorly understood.

Glycogen supercompensation. The phenomenon of glycogen supercompensation provides further evidence for the role of glycogen levels in dictating muscle energy metabolism and hormonal sensitivity. Glycogen supercompensation occurs following exercise and carbohydrate refeeding, when muscle glycogen stores are replenished to levels above that of the normal fed state. Glycogen supercompensation results in a transient insulin resistance that is corrected upon return of cellular glycogen levels to their physiological set point. Studies have shown that both insulin-stimulated and contraction-induced translocation of GLUT4 to the plasma membrane were reduced in glycogen-supercompensated muscle of rats, resulting in decreased glucose uptake (28). Furthermore, the reduction masked the adaptive increases in GLUT4 expression resulting from exercise training (22, 27, 28).

Conversely, Garcia-Roves et al. (15) examined the effects of preventing glycogen repletion on the postexercise GLUT4 increase. Rats were subjected to bouts of swimming exercise and then either fasted or fed normal or carbohydrate-free chow. In rats fed normal chow following exercise, elevated GLUT4 levels and insulin sensitivity returned to baseline levels within 42 h postexercise, after glycogen levels were restored. In contrast, in the rats fed carbohydrate-free food to prevent glycogen restoration, the increases in GLUT4 expression and insulin sensitivity were retained for at least 66 h. At this point, when fed carbohydrate-containing chow, the rats accumulated glycogen to a similar extent as rats fed normal chow immediately following exercise. In parallel, GLUT4 protein levels returned to baseline concentrations (15). Thus these data show that the adaptations in order to increase glucose transport after exercise will persist until glycogen levels are restored, underscoring the importance of glycogen stores in the physiological responses of skeletal muscle. However, the molecular mechanisms by which alteration of glycogen levels, and presumably modulation of metabolic fluxes into and out of glycogen stores, are translated into adaptive changes in muscle gene expression are not currently understood.

LIVER GLYCOGEN

Overview

Although the majority of postprandial plasma glucose is stored in skeletal muscle, hepatic glycogen makes up 10% of total liver weight when fully replete, reflecting the importance of glycogen metabolism in liver function. When plasma glucose levels rise after a meal, the liver clears glucose and stores it as glycogen. Conversely, when blood glucose concentrations fall during fasting or exercise, hepatic glycolytic rates drop due to decreased levels of the key glycolytic regulator fructose-2,6-biphosphatase. The liver produces glucose in response to an increase in the circulating glucagon-to-insulin ratio via mobilization of hepatic glycogen stores and increased expression of key gluconeogenic enzymes resulting in de novo synthesis of glucose from 3-carbon precursors. Thus hepatic glycogen metabolism plays a central role in maintaining circulating blood glucose levels around the physiological set point of 5 mM.

The differential functions for liver vs. muscle glycogen metabolism are highlighted by the tissue-specific enzymatic isoforms regulating glucose and glycogen metabolism. Muscle and liver express different isoforms of glycogen synthase and phosphorylase. Although their regulation by covalent modification is similar, each enzyme is differently sensitive to allosteric regulators, reflecting the metabolic demands of liver and muscle. Additionally, the major glucose transporter in liver is GLUT2, which, unlike GLUT4, is constitutively localized in the plasma membrane and possesses a relatively low affinity for glucose. Hepatocytes also express a specialized hexokinase, termed glucokinase, which is not subject to feedback inhibition by G-6-P. Similar to their function in the pancreatic β-cell, the kinetic properties of GLUT2 and glucokinase together act as a “glucose sensor” in which glucose uptake fluctuates in synchrony with changes in plasma glucose concentrations, enabling the regulated uptake and production of glucose as needed.

Hepatic Glycogen Metabolism in the Fasted State: Two Models of Hepatic Glucose Output

The liver provides glucose to the bloodstream during times of fast or increased energy demand through gluconeogenesis and the mobilization of intracellular glycogen stores. These pathways have been considered separate entities, each providing the G-6-P that is dephosphorylated and secreted from hepatocytes (Fig. 1A). However, recent work in several areas has suggested that gluconeogenesis and glycogen metabolism comprise a single interconnected metabolic circuit rather than two discrete arms (Fig. 1B). These results suggest that, as in muscle, hepatic glycogen metabolism occupies a key role in the coordination of cellular responses with nutrient status under a variety of physiological conditions.

Evidence for the interrelation of gluconeogenesis and glycogenolysis came from studies where human volunteers were infused with gluconeogenic precursors under insulin and glucagon clamps. It was hypothesized that an increased supply of gluconeogenic precursors would result in enhanced hepatic glucose output. Instead, researchers found that hepatic glucose production and plasma glucose concentrations were not significantly altered, suggesting that glycogen breakdown was downregulated when gluconeogenic precursor supply was increased (23). Similarly, inhibition of gluconeogenesis did not correct hyperglycemia in type 2 diabetic patients, again because glycogen breakdown was increased to maintain hepatic glucose output (50). The results of these studies suggested the hypothesis of “hepatic interregulation,” in which modulation of one arm of glucose production would be compensated for by changes in the other, thus maintaining hepatic glucose output at the same level. However, in 1998, Martin et al. (34) demonstrated that glycogenolysis could be reduced in vivo in a mouse model of diabetes by administration of a glycogen phosphorylase inhibitor, CP-91149. Unlike studies in which gluconeogenesis was inhibited, reduction of glycogenolysis resulted in decreased hepatic glucose output and improvement of hyperglycemia (34). Fosgerau et al. (14) reported similar results using another glycogen phosphorylase inhibitor, dianinoebenzidine, in a canine model. These studies demonstrated that changes in gluconeogenesis may result in alterations in glycogenolysis, but that the converse does not occur.
Precursors are metabolized to G-6-P through a continuous pathway (Fig. 1A) in which gluconeogenesis and glycogen metabolism form a highly interconnected pathway, even in the fed state (Fig. 1B). This model helps predict multiple layers of regulation and explains how inhibition of glycogen phosphorylase would result in decreased hepatic glucose production and plasma glucose levels.

**Hepatic Glycogen Metabolism in the Fed State: Glucose and Gluconeogenic Precursor Disposal in the Liver**

Further evidence in support of a continuous pathway for gluconeogenesis and glycogen metabolism comes from studies of nutrient disposal in the fed state. Glycogen may be synthesized from extracellular glucose (direct pathway) or from glucose derived from gluconeogenic substrates (indirect pathway). Although studies dating back to the 1930s showed that administration of labeled glucose through a single bolus resulted in the majority of hepatic glycogen being synthesized via the direct pathway, a growing body of evidence in the 1970s indicated that G-6-P from glucose was inefficiently used as the immediate precursor of liver glycogen. This apparent inconsistency was termed the “glucose paradox” (reviewed in Ref. 36).

To address the different outcomes of these studies, Newgard et al. (41) compared results from different experimental designs and evaluated which more closely approximated physiological conditions. In the older experiments, administration of the large intragastric bolus resulted in a nonphysiological hyperglycemia that presumably allowed glucose to enter the hepatocyte via GLUT2 and glucokinase at higher than normal rates. In the later studies, labeled glucose was infused intragastrically at a constant rate to achieve circulating glucose levels similar to those in rats refed following a fast. Under these conditions, less than one-third of liver glycogen was synthesized via the direct pathway. Additional evidence came from studies in humans by Shulman and colleagues (8, 58, 60), which indicated that gluconeogenic compounds are the major carbon source for hepatic glycogen. Finally, O’Doherty et al. (43) demonstrated that hepatic glycogen synthesis was increased in rats overexpressing PTG, a glycogen-targeting subunit of protein phosphatase-1. Although glucose incorporation into glycogen (direct pathway) was increased, the major mechanism for enhancement of glycogen synthesis was through increased incorporation of gluconeogenic precursors into glycogen (indirect pathway).

It is now accepted that the major source of glucose for hepatic glycogen stores is derived from 3-carbon compounds such as lactate, glycerol, and alanine and other gluconeogenic amino acids rather than directly from extracellular glucose. The synthesis of these compounds into glycogen comprises a system where gluconeogenesis and glycogen metabolism form a highly interconnected pathway, even in the fed state (Fig. 1B). Although these data represent a fairly recent paradigm shift, studies dating from the 1940s demonstrated that labeled carbon from gluconeogenic compounds could be tracked to glycogen (61, 70). To confirm these initial observations, the laboratory of Radziuk administered $^{14}$C- and $^3$H$\text{glucose}$ tracers orally or intravenously to human subjects to quantitate glycogen synthesis from glucose compared with gluconeogenic precursors. Data demonstrated 150% more deposition of labeled carbon from gluconeogenic precursors over that from glucose (51). Studies also suggest that, in addition to providing substrate for glycogen synthesis, gluconeogenic compounds help regulate glycogen metabolism (reviewed in Ref. 71). For example, several groups demonstrated that incubation of isolated hepatocytes with gluconeogenic amino acids resulted in increased activation of glycogen synthase (37, 40). Studies demonstrating feed-forward regulation of gluconeogenic compounds on glycogen synthesis provide additional evidence for the proposed model of a continuous metabolic pathway.

**Glycogen Cycling as a Key Regulator of Hepatic Function**

Under the proposed model, the funneling of gluconeogenic precursors and glucose through glycogen appears to generate a
futile cycle, in which glycogen synthesis and breakdown occur simultaneously. However, numerous studies demonstrate that glycogen cycling is a sensitively regulated circuit that allows the hepatocyte to be highly responsive to multiple stimuli. In a study of humans fasted for 60 h, as expected, hepatic glycogen levels were low, and gluconeogenesis accounted for the vast majority of hepatic glucose output (19). Subjects were then infused for 10 h with [13C]glycerol, followed by a pulse of glucagon, and glycogen stores were allowed to recover. Seventy-eight percent of the postglucagon glycogen was derived from the gluconeogenic precursor (19). These data indicate that glucose cycles through glycogen even when cellular stores are extremely low, which may prevent the complete depletion of cellular glycogen stores during prolonged fast.

Pharmacological inhibitors of glycogen phosphorylase have provided another critical tool to study the contribution of aberrant hepatic glucose output to hyperglycemia in diabetic patients, but also glucose cycling through glycogen (34). In primary hepatocytes from fasted rat, no net glycogen synthesis was detectable (35). However, both glycogen synthase and phosphorylase were in an activated state, and addition of a phosphorylase inhibitor promoted glycogen synthesis. Additionally, in a study of fasted dogs, addition of phosphorylase inhibitor resulted in accumulation of glucose derived from gluconeogenic precursors into glycogen (57). These studies provided further support to the notion that newly synthesized glucose cycles through glycogen routinely in the fasted state. Finally, treatment of primary hepatocytes with a phosphorylase inhibitor blocked conversion of [14C]lactate to [14C]glucose by over 50% and resulted in a threefold increase in [14C]lactate incorporation into glycogen (34). This unexpected result suggests that, not only does glycogen derived from gluconeogenesis cycle through glycogen, this step may be required for efficient dephosphorylation and secretion of newly synthesized glucose into the bloodstream (Fig. 1B).

Glucose cycling through glycogen also occurs in the fed state. By use of perfused rat livers under conditions favoring glycogen synthesis, tissues were pulsed with [13C]glucose and chased with [12C]glucose or [13C]glucose as a control (59). In the continued presence of [13C]glucose, there was an increase in nuclear magnetic resonance peak height in glycogen over time. However, during a [12C]glucose chase perfusion, there was a rapid decline in [13C] label content in glycogen, implying continual turnover in liver glycogen even under conditions favoring glycogen accumulation, since the [13C] label was briskly being lost from glycogen during the chase with [12C]glucose (59). These data also indicate a role for glycogen turnover to prevent overaccumulation of hepatic glycogen stores, which may be physiologically important due to expression of GLUT2 and glucokinase isoforms resulting in a continual flux of G-6-P into hepatocytes during prolonged hyperglycemia.

Finally, several studies have provided evidence that under certain experimental conditions glucose cycling through glycogen is negligible. In cells that had high glycogen stores and then were treated with a high ratio of glucagon to insulin, glycogen turnover was undetectable due to low synthetic rates (16). Additionally, in glycogen-depleted cells that were then stimulated with high glucose and insulin, glycogen degradation was markedly suppressed, enabling maximal rates of glycogen repletion (14). These results indicate that glucose cycling through glycogen is not constitutive but adapts to the metabolic requirements of hepatocytes.

A Model To Predict Glucose Flux Through Glycogen

The work on the interplay among hepatic glucose output, gluconeogenesis, and glycogen metabolism leads us to propose the model in Fig. 2, where rates of glucose cycling through glycogen are dependent on intracellular glycogen stores and extracellular signals. Three to four hours after a meal, as blood glucose levels start to fall, insulin secretion drops, leading to a decrease in the insulin/glucagon ratio and an increase in cytosolic cAMP concentration ([cAMP]). An initial burst of glycogen mobilization occurs in order to provide glucose for release into the bloodstream (Fig. 2, top left), and glucose cycling would be low due to high glycogen stores and low hepatic glucose production. As glycogen stores fall, the gluconeogenic component of hepatic glucose output would increase (Fig. 2, bottom left) as lactate and pyruvate are synthesized into G-6-P. Glucose cycling through glycogen would commence, in part to preserve glycogen stores. Potentially, the linkage of these pathways into one circuit would also provide a metabolic mechanism for the liver to keep track of total glucose production and enable the liver to balance energy demand by the body with its capacity to provide glucose from gluconeogenesis and glycogenolysis. When the fast is broken by a meal, insulin and glucose levels would rise, [cAMP], would fall (Fig. 2, x-axis, right), and glucose storage as glycogen would be favored. Initially, resynthesis of glycogen chains would be strongly favored, preventing glucose cycling (Fig. 2, bottom right). However, as glycogen levels grew, glycogen degradation and glucose cycling would commence to prevent overaccumulation of glycogen (Fig. 2, top right).

Conclusion

Classical studies of glycogen metabolism have figured prominently in the progress of biochemistry and signal transduction due to seminal discoveries in the areas of protein phosphorylation, intracellular second messengers, and phosphatase-targeting subunits. Following in that tradition, current
research has highlighted additional roles for glycogen in cellular physiology beyond its simple function as an energy depot. In muscle, where a variety of energy sources are utilized during contraction, rates of glycogen metabolism help orchestrate the changing integration of cellular fuel utilization pathways during exercise. In liver, glycogen turnover links gluconeogenesis into a single circuit to coordinate metabolically hepatic glucose output. These findings also generate new questions regarding glycogen metabolic function and regulation, ensuring that the study of glycogen will continue to be a dynamic, expanding field.

GRANTS

This work was supported in part by Grant R01 DK-064772 from the National Institute of Diabetes and Digestive and Kidney Diseases and by a Career Development Award from the American Diabetes Association.

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