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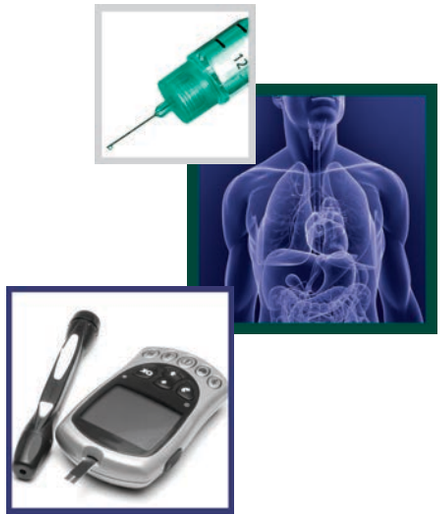

Regulation of glycogen synthase in muscle and its role in Type 2 diabetes

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Practice Points

- Glycogen synthase (GS) is regulated covalently via multiple phosphorylation sites and allosterically by glucose-6-phosphate.
- Physiological stimuli such as insulin, exercise and glycogen concentration affect GS activity.
- GS activity measurements are obtained *in vitro* and do not take into account localization of GS within the muscle cells.
- GS activity and glycogen synthesis are impaired in insulin-resistant subjects.
- Hyperglycemia can rescue glycogen synthesis rates in insulin-resistant subjects.
- Genetic mouse models suggest that glucose-6-phosphate flux overrides covalent regulation of GS activity.
- Impaired GS activity and glycogen synthesis are probably preceded by insulin resistance.
- GS is essential for normal glycogen levels, but not for normoglycemia.
- Exercise training improves glycogen synthesis rates in insulin-resistant subjects, but effects on GS activity are minor.
- Physical inactivity leads to reduced insulin sensitivity and GS activity.
- Acute exercise induces immediate postexercise GS activation and glycogen synthesis in healthy and insulin-resistant subjects.

SUMMARY Type 2 diabetic patients exhibit reduced insulin-stimulated glucose disposal rates along with impaired muscle glycogen synthase (GS) activity and glycogen synthesis. After a meal, muscle is an important glucose sink and a large proportion of glucose entering muscle is converted to glycogen. It is, therefore, a clinically relevant question to ask whether impaired GS activation and glycogen storage in muscle are defects responsible for reduced glucose disposal in Type 2 diabetes. This short review first provides a brief mechanistic background on regulation of GS activity and then presents evidence from human and rodent

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studies to discuss the possible role of dysregulated GS in the etiology of Type 2 diabetes. We conclude that impaired GS activity and glycogen synthesis in skeletal muscle of Type 2 diabetic patients is mainly a secondary manifestation of skeletal muscle insulin resistance of glucose transport.

In healthy individuals, blood glucose levels are tightly regulated. During fasting, glucose metabolism in the CNS and peripheral tissues is matched by production of glucose primarily by the liver, but also by the kidneys. After a meal, glucose-induced hyperinsulinemia along with hyperglycemia block endogenous glucose production and glucose is taken up in skeletal muscle [1,2]. As a result, plasma glucose levels return to baseline 1–3 h after a meal. Glucose enters muscle cells by facilitated diffusion. In response to insulin, vesicles containing the insulin-sensitive glucose transporter GLUT4 translocate from intracellular compartments to the plasma membrane and transverse tubuli (reviewed in 3]), thereby increasing the membrane permeability to glucose. Moreover, in the insulin-stimulated state a major part of glucose is stored as glycogen [4], which makes glycogen synthesis in muscles an important process for normal whole-body glucose disposal.

Skeletal muscle glycogen synthesis is a multistep reaction that converts glucose into its storage molecule, glycogen. Synthesis of glycogen is initiated by the autocatalytic protein glycogenin, but the rate by which glycogen synthesis occurs is controlled by uridine diphosphate glycosyltransferase glycogen synthase (GS) [5].

In insulin-resistant subjects normal plasma insulin concentration fails to induce normal whole-body and skeletal muscle glucose uptake [6]. Insulin resistance is a strong predictor for the development of Type 2 diabetes and can precede the disease by over 10 years [7,8]. Insulin-stimulated GS activity and glucose disposal rates in humans correlate strongly [9], and thus impaired GS activity mirrors the defect in glucose disposal and glycogen synthesis in insulin-resistant subjects [10,4]. It has, therefore, been speculated that dysfunction of GS may induce insulin resistance of glucose uptake. On the other hand, it has been argued that the decrease in glucose uptake due to insulin resistance limits the substrate supply for glycogen synthesis and that reduced GS activity is, therefore, a secondary manifestation reflecting this shortage [11]. This brief review aims to discuss what comes first, GS impairment or insulin resistance. In addition, the effects of chronic and acute exercise are addressed.

Regulation of skeletal muscle GS: interplay between covalent & allosteric inputs

Regulation of GS is complex and its covalent modification involves at least nine inhibitory phosphorylation sites, of which site 2, 2a, 3a and 3b are probably the most important for the regulation of GS activity **(Figure 1)** [5,12–14]. Importantly, GS has been reported to be hyperphosphorylated at sites 2 and 2a in Type 2 diabetic patients [15]. Allosteric regulation entails binding of glucose-6-phosphate (G6P) to GS, which increases activity and can supersede inhibitory phosphorylations [16,17]. Intramuscular glycogen content in itself may inversely affect GS activity [18–20]; although whether this is a direct allosteric effect is unknown. It is important to note that overall accretion of skeletal muscle glycogen is not only a function of glycogen synthesis, but also involves glycogenolysis. The rate of the latter is controlled by glycogen phosphorylase, which catalyzes the release of glucose-1-phosphate from the terminal α-1,4-glycosidic bond of the glycogen molecule [21]. While GS is generally active in the anabolic state (e.g., after a meal), glycogen phosphorylase is mainly activated in the catabolic state (e.g., during exercise).

Insulin covalently modifies GS activity via activation of Akt and PP1. Insulin-stimulated Akt activation leads to inactivation of GSK3 [22,23], thereby preventing GSK3 from phosphorylating GS **(Figure 2)** [24,25]. The dephosphorylated state is further facilitated by activation of PP1, leading to further activation of GS [26]. In addition to the covalent activation, insulin-stimulated glucose uptake increases the intramuscular G6P concentration leading to allosteric activation of GS [18,27].

During muscle contraction there is net glycogen breakdown, and net GS activity may increase or decrease during muscle contraction/exercise [28] depending on the relative strengths of signals with opposing actions on GS [28,29]. Exercise-related signals that probably modulate GS activity involve adrenaline and AMPK. Plasma adrenaline levels increase with exercise intensity [30]. Adrenaline binds to the muscle cell β2 receptors and activates PKA by elevating cyclic AMP levels. Increased PKA signaling inhibits PP1 and, thus, reduces the rate at which GS can be dephosphorylated.

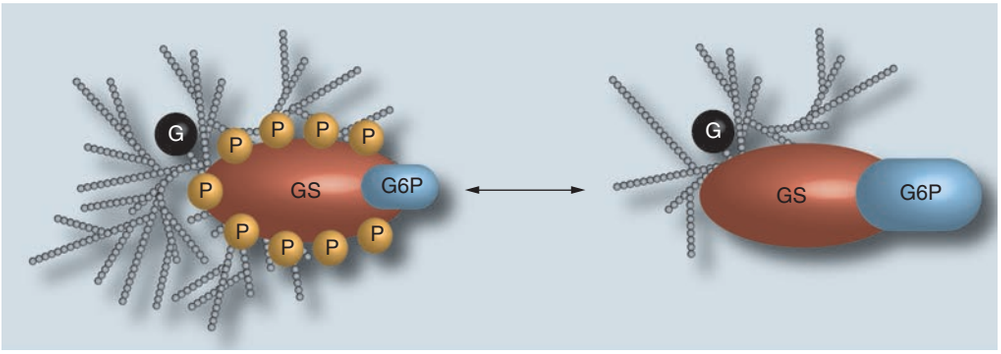

Figure 1. Covalent and allosteric regulation of glycogen synthase in skeletal muscle.
Hyperphosphorylation, low G6P and high glycogen concentrations lead to decreased GS activity.
Dephosphorylation, high G6P and low glycogen concentrations increase GS activity.
G: Glycogenin; G6P: Glucose-6-phosphate; GS: Glycogen synthase; P: Phosphorylation.

In addition, increased PKA activity also increases phopshorylation at site 2 on GS [31].

Muscle contractions activate AMPK [32], which has also been reported to phosphorylate muscle GS at site 2 *in vitro* and probably *in vivo* [33,34]. This is believed to be an inhibitory modification [35], because *in vitro* GS activity is reduced by AMPK-mediated site 2 phosphorylation [33], while increased GS activity has been reported in mice that lack the α2 subunit of AMPK hypophosphorylation on the site 2 residue [34].

Following exercise, however, increased GS activity is invariably found. To make matters more complicated, discordant phosphorylation patterns have been reported. In a time course experiment, Lai *et al.* found that *ex vivo* contractions of rat fast-twitch muscle led to phosphorylation of GS on site 2, while site 3a was dephosphorylated [36]. Site 2 phosphorylation has been associated with decreased GS activity [35,34], whereas site 3a is regulated by insulin and its dephosphorylation is reported to increase GS activity [25,14]. This illustrates the complex regulation of GS and the difficulties experienced when interpreting results.

It is important to note that GS activity is measured *in vitro* by its ability to convert uridine diphosphate glucose to glycogen. Total activity is determined by high levels of G6P (~5–10mM), which override any covalent control. GS activity can also be measured in the absence of G6P, which reflects the covalent impact (I-form), and in the presence of low levels of G6P (0.1–0.2 mmol/l; fractional velocity), which are believed to emulate physiological *in vivo* conditions. In fasting humans, G6P concentrations are approximately 0.12 mmol/kg muscle [37]. Both I-form and fractional velocity are related to total GS activity and reported as a percentage of total activity. This approach makes the assumption that there is a homogenous G6P concentration throughout the muscle cell and does not take into account the role of localization of GS within the muscle cell in response to different stimuli [19,38].

Insulin resistance & GS: evidence from glucose & insulin clamp studies

Using nuclear magnetic resonance spectroscopy, Shulman *et al.* demonstrated that glycogen synthesis accounts for most of the whole body glucose disposal during a hyperglycemic-hyperinsulinemic clamp and is severely impaired in insulin-resistant individuals [4]. After ingestion of a mixed meal, the muscle glycogen storage defect persists in Type 2 diabetics [39], but following a meal challenge most of the glucose is stored as liver glycogen [2,40]. Liver glycogen synthesis is also impaired in Type 2 diabetics [41] (hepatic glucose uptake and storage are reviewed in [42]). Regarding muscle GS, if a defect in GS activity is solely responsible for decreased glycogen synthesis and, in turn, insulin resistance of glucose uptake, intramuscular G6P would accumulate. However, Rothman *et al.* demonstrated that G6P increments during a hyperglycemic-hyperinsulinemic clamp were lower in Type 2 diabetics, which suggests that impaired glucose uptake is responsible for insulin resistance [37]. The same G6P defect along with insulin resistance was present in healthy offspring from diabetic parents, who have a genetic predisposition to developing Type 2 diabetes [43]. These findings indicate that insulin resistance, measured as impaired glucose uptake, precedes the onset of

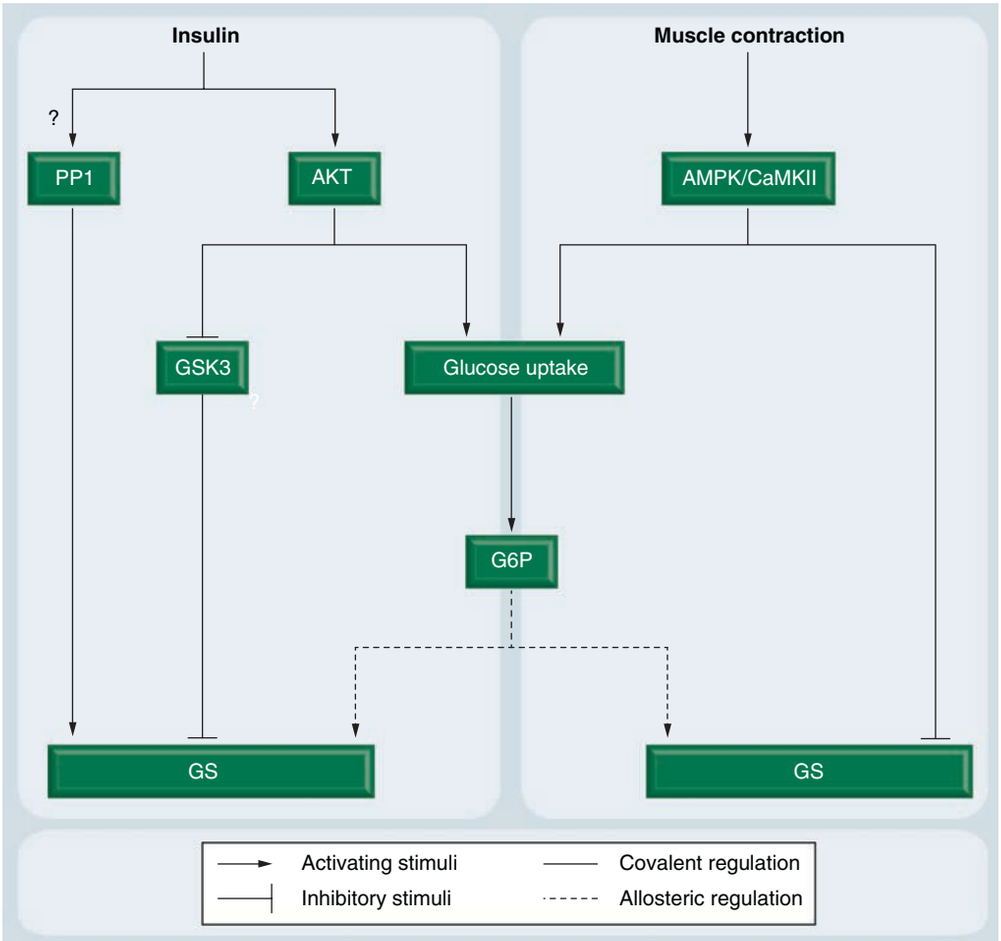

Figure 2. Regulation of glycogen synthase activity by insulin and muscle contraction. Insulin stimulation increases GS activity covalently and allosterically. Muscle contractions activate AMPK and CaMKII (for review see [81]). During muscle contractions, GS integrates inhibitory covalent modifications and stimulatory G6P binding.
CaMKII: Ca^{2+}/calmodulin-dependent kinase II; G6P: Glucose-6-phosphate; GS: Glycogen synthase.

Type 2 diabetes, which has also been suggested by longitudinal studies [7,8]. In support of this, a recent report by Vind *et al.* demonstrated that, in contrast to when they were clamped at normal fasting glucose levels (~5.5 mmol/l; euglycemic), when Type 2 diabetic subjects were clamped at their respective fasting glucose levels (~9 mmol/l; isoglycemic) glucose disposal and nonoxidative glucose metabolism (glycogen synthesis) were similar to weight- and age-matched nondiabetic controls clamped at their respective fasting levels [44]. The idea that hyperglycemia can normalize impaired glucose metabolism as determined by a euglycemic clamp is not new [45,46], but Vind *et al.* compared signaling events in Type 2 diabetics with the euglycemic and isoglycemic clamps and found that upstream signaling, including Akt phosphorylation, Akt2 activity, GS activity and dephosphorylation of sites 2 and 2a, was impaired compared with controls. This signaling defect was not rescued during the isoglycemic trial. This implies that the normalization of whole-body glucose disposal and nonoxidative metabolism are caused by the mass action of raising plasma glucose levels. The clinical implication of this is that glucose delivery into the muscle cell is probably an important determinant of insulin-stimulated glycogen synthesis following an overnight fast. Whether this conclusion applies during conditions other than the overnight fasted state is uncertain.

In concurrence, allosteric regulation of GS by G6P has been suggested to override covalent regulation even *in vivo* [47,48]. In mice expressing

constitutively active GSK3 [48], insulin was unable to induce dephosphorylation of GS. Therefore, insulin-stimulated GS activity was decreased, but *in vivo* glycogen synthesis rates and glycogen levels were unperturbed. This indicates that covalent regulation is trumped by allosteric sensing of normal G6P levels. In agreement with this, Bouskila *et al.* described a mouse variant in which the G6P-sensing domain of GS was mutated to a G6P insensitive domain [47]. Strikingly, this mutation reduced glycogen synthesis by 80%, despite the fact that insulin-induced GS dephosphorylation was maintained. This provides evidence that glucose uptake, rather than covalent regulation of GS is rate limiting for glycogen synthesis. One caveat is that although mouse models are useful, results found using mice are not always applicable to human physiology. For example, while the majority of the glucose entering muscle in the absorptive state in humans is converted to muscle glycogen, the mouse only stores approximately 25% of that glucose as glycogen [47]. In addition, the glycogen concentration in mouse muscle is only approximately 10% of the concentration in human muscle (reviewed in [49]). However, it is noteworthy that these G6P-insensitive mice had normal insulin-stimulated glucose metabolism, as evidenced by normal whole-body glucose disposal during a euglycemic/hyperinsulinemic clamp [47]. This demonstrates that a defect in GS and glycogen storage does not necessarily lead to insulin resistance of glucose uptake.

Insulin resistance & GS: evidence from human genetic variants

To determine the role of GS in insulin resistance and subsequently Type 2 diabetes, gene variants affecting GS have been investigated. In Finnish subjects, Groop *et al.* found a polymorphism in the muscle GS gene (*GYS1*) that was more prevalent in Type 2 diabetics (30%) compared with nondiabetic controls (8%), and thus, identified a possible genetic risk factor [50]. Furthermore, within the Type 2 diabetic group, rates of glucose disposal and glycogen synthesis were significantly lower in the subjects harboring the polymorphism in the GS gene [50]. Although this implicates GS-dependent impairment of glucose disposal, it is difficult to draw such a conclusion, because neither GS activity nor intramuscular G6P levels were measured and the genetic defect could possibly be accompanied by other unrecognized defects that affect the risk of developing insulin resistance. Interestingly, protein expression of GS was the same in the Type 2 diabetic subjects without the polymorphism compared with the Type 2 diabetics with the polymorphism. Moreover, while a similar study confirmed that the polymorphism may be used as a Type 2 diabetes risk marker in the Danish population [51], the findings are unsubstantiated in other populations [52]. None of these *GYS1* studies investigated whether there was a genotype effect on GS activity or glycogen levels, which would have provided clues as to how important GS is to whole body glucose metabolism.

Another genetic variant affecting GS was presented by Kollberg *et al.* in a case report describing three siblings that were homozygous for a stop mutation in *GYS1* [53]. No GS protein could be measured by western blot in cardiac or skeletal muscles from these three patients. Moreover, extremely low muscle glycogen levels were reported. This is important evidence from humans that GS is indispensable for glycogen synthesis in muscle. Unfortunately for this discussion, parameters of insulin-regulated glucose metabolism were not measured. The major clinical outcomes reported were severe exercise intolerance and cardiomyopathy.

Savage *et al.* described a loss-of-function variant in the human *PPP1R3A* gene, which encodes a part of the PP1 protein [54]. Normal PP1 dephosphorylates and, thus, activates GS upon insulin stimulation. Heterozygous carriers of this mutation displayed lower basal and post-prandial glycogen levels compared with other nondiabetic controls. Basal glycogen levels were even lower than in Type 2 diabetic controls. Despite this marked glycogen defect, plasma glucose and insulin levels were similar in the nondiabetic participants and the subjects with the *PPP1R3A* variant following a meal challenge. In mice with the same *PPP1R3A* gene mutation GS activity was impaired and glycogen levels were decreased compared with wild-type control mice. However, similar to the human phenotype, these mutant mice were glucose tolerant and insulin sensitive. In two other mouse models in which PP1 function was abrogated in skeletal muscle, GS activity and glycogen were also markedly decreased, but while Delibegovic *et al.* reported that the mice became glucose intolerant and insulin resistant at the age of 12 months [55], Suzuki *et al.* describe normal glucose tolerance and insulin sensitivity in their mice [56]. Mice lacking muscle GS caused by disruption of the *GYS1* gene also

have extremely low muscle glycogen levels, but whole-body glucose homeostasis is normal [57].

To distinguish between inherited and acquired defects in insulin-mediated GS activity Huang *et al.* studied monozygotic twin pairs discordant for Type 2 diabetes and controls matched to the nondiabetic twin [58]. They found that insulin-stimulated GS activity in muscle was impaired in the diabetic twins compared with the nondiabetic twins and healthy controls. Since no significant difference in GS activation was found between nondiabetic twins and controls, Huang *et al.* concluded that the defect in GS activity in the diabetic twins was acquired and secondary to hyperglycemia [58].

Taken together, these studies imply that GS is necessary for normal glycogen synthesis and glycogen levels, but GS is not an invariable determinant of insulin sensitivity of glucose uptake. It appears that insulin resistance of glucose transport precedes impaired GS activity in the development of Type 2 diabetes.

Insulin resistance & GS: effects of exercise

Perseghin *et al.* demonstrated that aerobic exercise training improves insulin-stimulated whole-body glucose metabolism and glycogen synthesis in insulin-resistant subjects to the same extent as in nondiabetic controls [59]. Before training, G6P appearance in muscle in response to insulin was lower in the Type 2 diabetics, which indicates a defect in muscle glucose uptake. After training, G6P appearance had normalized and was similar in both groups, suggesting that training had normalized the effect of insulin on glucose transport. Unfortunately, GS activity was not measured. However, two recent studies have also demonstrated the positive effect of aerobic exercise training on whole-body glucose disposal and glycogen storage, but in Type 2 diabetics [60,61]. GS activity was assessed, but the authors found no improvement in the activation of GS with insulin. In addition, exercise training led to increased expression of GLUT4 and Akt proteins, which likely contribute to the improvement in whole-body glucose metabolism [60,61].

Physical inactivity and bed rest decrease insulin sensitivity [62,63]. In a recent study 7 days of bed rest resulted in a reduced whole-body glucose disposal rate, reduced glucose extraction by muscle, decreased GS activity, and GLUT4 and Hexokinase protein content [64]. Although this demonstrates that physical inactivity impacts glucose metabolism and GS activity, it is difficult to identify one point of failure that accounts for the impaired glucose extraction by the leg muscles.

One session of physical activity improves whole-body glucose disposal [65,66], an effect mediated by the increased insulin sensitivity of the exercised muscles [67–69]. Similarly, it has been demonstrated using a one-legged kicking protocol that GS activity increased in the exercised leg compared with the rested leg postexercise [67,70]. This suggests that local factors are responsible for the increase in GS activity and lower glycogen levels have also been implicated [19,71]. Whether the increased GS activity is important for the increased insulin sensitivity of glucose uptake after exercise is difficult to determine. In rats, increased insulin sensitivity of glucose uptake after exercise is ascribed to increased GLUT4 translocation after exercise [72]; however, the increase in GS activity is probably of importance for inducing glycogen synthesis rather than glucose oxidation. Recently, it was found that after an intense cycling bout, changes in GS phosphorylation and activity were similar between obese Type 2 diabetics and glucose-tolerant obese and nonobese subjects [73]. This suggests that analogous to the regulation of GLUT4, exercise can modulate GS activity independently of insulin signaling, and this effect is preserved in insulin-resistant subjects.

In support of this, Price *et al.* found that glycogen-depleting exercise resulted in similarly robust glycogen synthesis rates in both insulin-resistant and control subjects within the first hour of recovery [74]. Since this initial recovery phase has been defined as the insulin-independent phase [75,76], it fits that in the subsequent insulin-dependent phase, glycogen synthesis rates between 1 and 5 h postexercise were markedly lower in the insulin-resistant individuals. What explains the initial normal rate of glycogen synthesis? While GLUT4 translocation [77,78] and glucose uptake in response to insulin is impaired in insulin-resistant subjects [79], exercise-mediated GLUT4 translocation is normal [80]. Therefore, it has been argued that residual exercise effects in combination with low glycogen levels [19,71] mediate most of the glucose transport and subsequent glycogen synthesis in the immediate postexercise period [76]. With time, the exercise-induced membrane permeability decreases, and insulin becomes the main regulator of glucose uptake and glycogen synthesis rates [75]. This interpretation is supported by similar intramuscular G6P levels straight after

exercise in controls and insulin-resistant subjects, indicating similar rates of glucose uptake [74].

Together, these studies suggest that impaired glycogen synthesis rates in insulin-resistant subjects can be improved with exercise training. Whether this is a reflection of improved GS activity or improved substrate delivery to the enzyme is uncertain. In addition, acute exercise-induced GS activity is normal in insulin-resistant subjects, along with normal glycogen synthesis rates in the insulin-independent early postexercise phase.

Conclusion

With regard to the etiology of Type 2 diabetes, there is mounting evidence that impaired glycogen synthesis in skeletal muscle may be a secondary manifestation, preceded by skeletal muscle insulin resistance of glucose transport. It is likely that reduced glycogen synthesis is primarily a reflection of reduced glucose uptake by muscles, which is supported by the observation that glycogen synthesis is normal in insulin-resistant subjects immediately after exercise. Treatments aiming to alleviate insulin resistance in skeletal muscle should focus on the glucose uptake step, because in light of the present evidence, this should concomitantly improve glycogen synthesis rates. Importantly, exercise training improves whole-body insulin sensitivity along with improved glycogen synthesis rates in both healthy and insulin-resistant subjects.

Future perspective

Although insulin resistance of glucose uptake and GS activity are seemingly entangled, it may not be clinically relevant to define what comes first. Interestingly, acute exercise appears to momentarily improve not only glucose uptake, but also GS dysfunction and glycogen synthesis in Type 2 diabetics. Further research exploring the molecular mechanisms behind the effect of physical activity may strengthen the argument that exercise as a lifestyle choice helps prevent and treat Type 2 diabetes. In addition, it could yield pharmacological targets that are independent of the insulin signaling pathway to help treat Type 2 diabetes. Researchers, however, must widen their focus and include the glucose uptake step, GS function and glycogen synthesis to yield clinically valuable information. Furthermore, lifestyle modifications such as type of diet or caloric restriction and their effect on glycogen metabolism in Type 2 diabetics need to be more extensively studied. Research investigating localization of glycogen particles and GS within the muscle cell, using various microscopy techniques, merits more attention because glycogen is not an evenly distributed homogenous mass in a muscle cell, but rather exists in granules within distinct locations. Similarly, GS is heterogeneously localized. Currently, we know little about how localization affects GS activity, but accounting for localization probably produces a more accurate snapshot of GS activity than the standard *in vitro* activity method from homogenized muscle.

Financial & competing interests disclosure

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■■ **Emphasizes the concept that a muscle cell has distinct compartments and cannot be viewed as a homogeneous entity. It also demonstrates the influence of glycogen content on glycogen synthase (GS) activity.**

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