Progresses of the Past Decade on Factors Contributing to Skeletal Muscle Glycogen Synthase Regulation

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Abstract

Glycogen is the primary storage form of glucose, which is metabolized actively in the liver and skeletal muscle of mammals. The skeletal muscle glycogen is used intracellularly to provide starting substrate for glycolysis, whereas liver glycogen is used mainly for the maintenance of blood glucose homeostasis. The conversion of glucose to glycogen is an ordered process, in which the enzyme glycogen synthase plays an essential role. The key events in the synthesis of glycogen have been elucidated in both the liver and muscle. The process of understanding the regulatory mechanism of glycogen synthase activity has led to the discoveries of many important players in cellular metabolism. Research in identifying factors modulating muscle glycogen synthesis has been of popular interest within the past decade. The goal of this review is to summarize potential factors affecting skeletal muscle glycogen synthase expression level and activity in literatures published for the past decade.

Keywords: Glycogen; Glycogen synthase; Glycogen phosphorylase; Insulin; Metabolic disorders; Skeletal muscle

Introduction

The ability to acquire and store energy is essential for an organism to face environmental challenges such as the lack of readily available nutrients. Glycogen, the highly branched polysaccharide, serves as the primary storage form of glucose [1]. The liver and skeletal muscles are the major storage sites of glycogen in human body. As shown in Figure 1, glycogen in the liver and skeletal muscle is used differently [2]. The role of glycogen within the skeletal muscle is to provide glucose for energy production while the primary role of glycogen in the liver is to supply glucose in the blood for the use in other tissues [2]. During the fed-state, the entry of dietary nutrients into the blood leads to the secretion of the anabolic hormone, insulin, from the pancreatic β-cells [2]. The activation of insulin signaling pathway increases the activities of enzymes involved in glycogenesis including Glycogen Synthase (GS), and in turn, enhance glycojenesis in hepatocytes and muscle cells [3,4]. During a fasting state, the entry of dietary nutrients into blood stops, and the pancreas reduces insulin and increases glucagon secretion, a process that reduces glycogenesis and enhances glycogenolysis [2]. GS and Glycogen Phosphorylase (GP) are primary enzymes for glycogen synthesis and glycojenolysis, respectively [5]. The hepatic glycojenolysis provides source of glucose to be released into the blood [5]. Unlike the liver, muscle lacks glucose-6-phosphatase activity which results in the use of glucose inside muscle cells [6].

The conversion of glucose to glycogen is an ordered process that has been studied extensively. UTP-glucose-1-phosphate uridylyltransferase catalyzes the conversion of glucose-1-phosphate to Uridine Diphosphate (UDP)-glucose in the expenditure of Uridine Triphosphate (UTP) [7]. At the core of the glycogen molecule is a protein homodimer known as glycogenin. Glycogenin has two tyrosine residues that will accept glucose moiety from UDP-glucose [3]. Therefore, glycogenin serves two roles in the process of glycogen synthesis: 1) the anchoring core molecule for glycogen formation, and 2) the initiating enzyme to begin the growing glycogen chain. UDP released from the reaction is recycled for the reformation of UTP. Glucose molecules are first linked by α(1→4) glycosidic bonds until the formation of a branch [3]. After roughly eight to ten glucose units have been linked via α(1→4) glycosidic linkage, the enzyme GS takes over the elongation process. After the glycogen molecule grows in length, the glycogen branching enzyme will catalyze the transfer of a terminal fragment of six to ten glucose moieties from a non-reducing end to the C-6 hydroxyl group of a glucose moiety closer to the interior of the glycogen molecule, forming an α(1→6) glycosidic bond [7,8]. With the increase of branching points in glycogen particles, it allows more non-reducing end for hydrolysis by GP at the same time and increases particle’s solubility, which helps more efficient glucose storage [8].

As shown in Figure 1, the activities of GS and GP are regulated covalently and allosterically. The activities of GS and GP are regulated in the liver and skeletal muscle covalently through phosphorylation and through allosteric effectors such as intracellular Glucose-6-Phosphate (G6P) and ATP levels [4,9]. G6P can either be used to enter the glycolytic pathway or converted to Glucose-1-Phosphate (G1P) by phosphoglucomutase [7]. Intracellular increases in G6P will assist in the activation of GS and inactivation of GP, therefore, favoring glycogen synthesis [7]. During periods of elevated energy (ATP) levels, GP will remain dephosphorylated in favor of glycogen formation [10]. Within skeletal muscle, when Adenosine Monophosphate (AMP) levels rise due to factors such as exercise, GP is phosphorylated to promote the formation of G1P, which can then enter the glycolytic pathway [10].

Glycogenesis and glycogenolysis are regulated by catabolic and anabolic hormones. The anabolic hormone insulin initiates its action after binding to the insulin receptor, a process that starts an intracellular signaling cascade. The binding of insulin and its receptor brings closer of the two insulin receptor β-subunit, which is a tyrosine kinase, and leads to auto-phosphorylation of tyrosine residues on each other [11]. Subsequently, activated β-subunits phosphorylate Insulin Receptor Substrate (IRS) proteins, with IRS-1 being the primary IRS in skeletal muscle, which recruits and activates Phosphatidylinositol-
3-Kinase (PI3K) [11]. Activated PI3K catalyzes the conversion of the membrane-associated Phosphatidylinositol-4,5-bisphosphate (PIP2) and ATP into Phosphatidylinositol-3,4,5-triphosphate (PIP3) and ADP. The increases in PIP3 leads to the recruitment of Akt, a serine/threonine protein kinase also referred to as Protein Kinase B (PKB), by interacting with its pleckstrin homology domain [12]. PIP3 also recruits PIP3-bound Phosphoinositide-Dependent Protein Kinase-1 (PDK1) to the plasma membrane which phosphorylates Akt at Thr308 [13,14]. The mammalian Target of Rapamycin Complex (mTORC2) will phosphorylate Akt at Ser473 leading to its activation [15]. In muscle cells, the insulin stimulation leads to an increase in amount of Glucose Transporter 4 (GLUT4) on the cell membrane, which is translocated from intracellular storage vesicles [16]. Akt will inactivate Glycogen Synthase Kinase-3 (GSK-3) by phosphorylating specific sites on the enzyme [17,18]. By inhibiting GSK-3, Glycogen Synthase Phosphatase (GSP) can activate GS by dephosphorylation [13,19]. The insulin signaling cascade is also associated with the activation of Protein Phosphatase 1 (PP1) which will lead to the dephosphorylation and activation of GS while inactivating GP, favoring glycogen formation [20].

Adrenaline, also referred to as epinephrine, and glucagon are catabolic hormones that stimulate an intracellular signaling cascade to promote glycogenolysis. Upon their secretion, glucagon binds to G-Protein Coupled Receptors (GPCRs) located on the surface of liver cells, whereas, adrenaline will bind to GPCRs on the surface of liver and muscle cells. The binding results in a conformation change of GPCR that leads to the Gs subunit of the stimulated G protein complex to exchange Guanine Diphosphate (GDP) for Guanine Triphosphate (GTP) and its release from the heterotrimeric complex [21]. The activated Gs will activate adenylyl cyclase, which, in turn, catalyzes the conversion of ATP into cyclic Adenosine Monophosphate (cAMP). The increase in cAMP level will activate Protein Kinase A (PKA) [21]. PKA is a complex that encompasses two regulatory subunits and two catalytic subunits. Once cAMP binds to the regulatory subunits, the complex dissociates, allowing the catalytic subunits of PKA to interact with and phosphorylate specific residues on a number of proteins [22]. Phosphorylation kinase and GS are two such proteins. The phosphorylation and activation of Phosphorylase Kinase by PKA will lead to the activation of GP, which results in glycogen breakdown [23]. PKA will also inhibit the acetylation of GP, which decreases PPI’s ability to bind to, dephosphorylate, and inactivate GP [24]. The phosphorylation of GS by PKA will result in reducing its activity, thus abrogating glycogen synthesis [25].

As insulin regulates the activities of enzymes for glycogenesis and glycogenolysis, glycogen metabolism is altered in diabetes [26]. There are numerous other conditions that can arise from or be associated with irregularities in skeletal muscle glycogen regulation [27]. The key events in the synthesis of glycogen have been well characterized, and factors affecting muscle glycogen synthesis have drawn attention for the past decade. The focus of this review is to identify factors that can potentially promote or abrogate the activities of GS as demonstrated by scholarly articles published within the past 10 years.

Studies were identified by searching the PubMed electronic database from June 2015 to February 2016. The search terms included were: skeletal muscle, glycogen, glycogen synthase. Time constraints were set on the date of publication with January, 2005-February, 2016 being eligible for inclusion. Only articles published in English were reviewed. Identified abstracts were screened for duplicates. Abstracts identified were reviewed and full articles for abstracts meeting criteria were retrieved and further evaluated.

Only original research studies reporting the influence of a manipulation that either decreases or increases in GS gene expression, protein level, and/or GS activity in which statistical significance was reported, were considered for inclusion. Both in vivo and in vitro study designs were eligible for inclusion. There was no restriction on type of subjects involved in in vivo studies to be included in this review. There was no restriction on length of experimental manipulation to be included in the review.

For the included studies, general study characteristics (author, year of publication, study design, comparison groups, and testing duration), characteristics of study population (for humans-age, gender, disease state; for animals-species, strain, disease state), experimental manipulation (exercise, diet, compound treatment), and study outcomes (regulation of GS gene expression, protein levels, and/or activity) were extracted from the included studies. MG independently extracted data from each study. Discrepancies regarding data extraction in studies were resolved by discussion with GC.

All extracted studies were divided into two categories based upon the study outcomes: promotion or abrogation of GS regulation. All identified studies had to report on at least one of the following outcomes: promotion or abrogation of GS gene expression, protein level, and/or activity. Studies that reported only on upstream or downstream factors of GS, but did not specifically report significant findings on GS were excluded from this review.

Factors Promoting GS Regulation

Factors leading to the positive regulation of GS have been of much interest within the past decade in both human (Table 1) and animal (Table 2) model systems.

Behavior and physical activity

As shown in Table 1, a study investigating the effects of a 6-month weight loss and aerobic activity program in elderly insulin-resistant men found that glucose utilization in them improved [28]. This is most likely due to the reported increase in insulin-induced GS activity [28]. Similar results were seen in rat skeletal muscle, in which insulin and muscle contraction have an additive effect on induction of GS activity
### Table 1: Factors positively impacting GS regulation in studies using human materials.

| Factor/Manipulation | Participants/Model System | Treatment/Manipulation; Duration | Findings | Proposed Mechanisms | Citation |
|---------------------|----------------------------|---------------------------------|----------|---------------------|----------|
| Physical Activity/Muscle Contraction | Obese subjects with or without T2DM (examined vastus lateralis muscle) | On a cycle ergometer for 60 min at an intensity (~70% VO\textsubscript{2max}) followed by 1-3 h of recovery period | ↑ GS activity ↓ GS phosphorylation at Ser7/10/640/644 | Exercise increases substrate affinity and decreases GSK-3β activity. | 30 |
| Lean and obese subjects with normal glucose tolerance, obese subjects with T2DM (examined vastus lateralis muscle) | On a cycle ergometer for 40 min at an intensity (~70% VO\textsubscript{2max}) | ↓ Glycogen content and GS phosphorylation at Ser7/641/645/649/653/657 | Exercise increases GS affinity for UDP-glucose and decreases GS phosphorylation at Ser7/641/645/649/653/657. | 31 |
| Obese subjects with or without T2DM (examined vastus lateralis muscle) | On a cycle ergometer with four to five sessions of 20 to 35 min per week at an average intensity (~65% VO\textsubscript{2max}) - 10 weeks | ↑ GS activity and GS protein level | Exercise can increase TBC1D4 signaling and influence key insulin-signaling protein levels. | 32 |
| Healthy, elderly male subjects (examined vastus lateralis muscle) | On cycle ergometers twice per week, strength and mobility training once per week and a 5 km walk once a week. 8 weeks | ↑ Glycogen content and GS protein level ↓ GS phosphorylation at Ser7/10/640 | Exercise enhances glucose uptake and decreases GS phosphorylation (Ser7/10/640) phosphorylation. | 33 |
| Middle-aged sedentary or regularly active subjects (examined vastus lateralis muscle) | Male subjects who performed endurance training (running and cycling) must have done so for years previous to study. Duration: 4 to 7 days between screening day and experimental (biopsy) day | ↑ GS protein level | Mechanism not described in detail, however, having a high VO\textsubscript{2max} may influence insulin sensitivity in muscle. | 34 |
| Calorie Restriction+Physical Activity | Older, overweight/obese insulin-resistant subjects (examined vastus lateralis muscle) | Aerobic exercise training (treadmill at 50-70% VO\textsubscript{2max}) for 20 to 50 min + weight loss (restrict intake by 500 calories/day). 6 months | ↑ GS activity (vs. baseline) | Calorie restriction+physical activity may enhance whole-body insulin sensitivity. | 28 |
| Healthy, overweight, or obese post-menopausal women with normal or impaired glucose tolerance (examined vastus lateralis muscle) | Women exercised at >85% heart rate reserve for 45 min, 3x per week and/or were instructed to reduce their caloric intake by 500 kcal/day. 6 months | ↑ GS activity (in women with impaired glucose tolerance who exercised and restricted calories vs. restricted calories alone) | Calorie restriction+physical activity may enhance whole-body insulin sensitivity. | 35 |
| GSK-3α Repression | Human skeletal muscle cells isolated from nondiabetic subjects (biopsy samples of the vastus lateralis) | Antisense oligo against GSK-3α were used for 6 h to knockdown expression in differentiating cells. Insulin (33 nM) for 30 min. | ↑ insulin-stimulated GS fractional velocity | GSK-3α repression may enhance insulin actions while overexpression can lead to insulin signaling impairment. | 41 |
| GSK-3β Repression | Human skeletal muscle cells obtained from nondiabetic subjects (biopsy samples of the vastus lateralis) | Transfection of cultured muscle cells with siRNA against GSK-3β for 4 h. Insulin (33 nM) for 30 min. | ↑ basal and insulin-stimulated GS fractional velocity | GSK-3β expression can affect GS activity in both basal and insulin-stimulated state, with GSK-3β repression allowing for activation of GS. | 43 |
| IL-6 | Human skeletal muscle cells obtained from nondiabetic subjects (biopsy samples of rectus abdominus) | IL-6 (5, 25, or 100 ng/mL). treatment for 20 min, 3 h, or 8 days | ↑ Glycogen synthesis (IL-6 ≥ 5 ng/mL) ↑ GS mRNA expression (IL-6 25 ng/mL, 8 days) | L-6 increases glycogen synthesis via a PI3-kinase-dependent mechanism. | 47 |
| Adiponectin | Lean subjects, obese subjects with or without T2DM (examined vastus lateralis muscle) | Euglycemic-hyperinsulinemic clamp performed for 4 h | ↑ insulin-stimulated GS fractional velocity (positive association with plasma adiponectin levels) | Plasma adiponectin may improve the ability to switch from lipid to glucose oxidation, and enhance muscle insulin sensitivity. | 48 |
| Pioglitazone | Obese women with PCOS (examined vastus lateralis muscle) | Euglycemic-hyperinsulinemic clamp performed for 3 h. Treatment with either pioglitazone (30 mg) or placebo once daily - 16 weeks | ↑ insulin-stimulated GS activity and dephosphorylation of GS atSer7/640/644 | Pioglitazone enhances insulin sensitivity and GS activity by promoting insulin-mediated dephosphorylation of GS at Ser7/640/644. | 57 |

**Abbreviations:** GS=Glycogen Synthase, GSK=Glycogen Synthase Kinase, IL-6=Interleukin-6, mRNA=messenger Ribonucleic Acid, PCOS=Poly cystic Ovary Syndrome, T2DM=type 2 Diabetes Mellitus, TBC1D4=Tre-2/USP6, BUB2, Cdc16 domain family, member 4, UDP=Uridine Diphosphate, ↑=significant increases due to factor/manipulation, ↓=significant decreases due to factor/manipulation.
| Factor/Manipulation | Participants/Model System | Treatment/Manipulation; Duration | Findings | Proposed Mechanisms | Citation |
|---------------------|---------------------------|---------------------------------|----------|---------------------|----------|
| Physical Activity/Muscle Contraction | Male Wistar rats (examined epitrochlearis muscle) | Muscles were kept rested or contracted electrically while incubating with or without insulin (10 mM/ML) for 30 min. | ↑ insulin-stimulated GS activity ↓ GS phosphorylation at Ser645/649/653/657 | Muscle contraction can increase AMPKα2 phosphorylation at Thr172 and decrease GS phosphorylation at Ser645/649/653/657. | 29 |
| | Female Sprague Dawley rats (examined triceps and epitrochlearis muscles) | Two groups- rats in the untrained group were housed individually in plastic cages and rats in the trained group were housed in wheel cages. Trained either for 1 week, 4 weeks, or 7 weeks. | ↑ Glycogen content after 4 and 7 weeks (triceps) ↑ GS activity after 4 and 7 weeks (triceps) | Physical activity may enhance insulin sensitivity and PP1 activity. | 36 |
| | Female white New Zealand rabbits (examined tibialis anterior muscle) | Electrostimulations to mimic contraction were applied for 1 h or 24 h. After stimulation, animals were allowed to recover for 0, 1, 6, 12, or 24 h. | ↑ Glycogen content during a 6 h recovery phase after a 24 h stimulation ↑ GS activity ↓ GP activity and GS (Ser640) phosphorylation | Prolonged muscle contraction can increase GS activity by decreasing GS phosphorylation at Ser640 while decreasing GP activity. | 37 |
| GLUT4 Repression | Wild-type and muscle-specific GLUT4-knockout mice Parent strains - 129SV and C57Bl/6J mice (examined soleus, gastrocnemius, and tibialis anterior muscles) | Transgenic mice with muscle-specific GLUT4 knock out. Electrical stimulation to mimic contraction. Mice were studied at 4 to 12 months of age. | ↑ Glycogen content ↑ GS activity (tibialis anterior and gastrocnemius) ↓ GS phosphorylation at Ser641 (gastrocnemius) ↓ GP activity (tibialis anterior and gastrocnemius) | Decreased muscle glucose transport was associated with decreasing GS phosphorylation at Ser641 and GP activity. | 38 |
| AMPK overexpression | Wild-type and transgenic mice with AMPKγ3 or AMPKγ3 R225Q mutant overexpressions Parent strain - FVB mice (examined EDL, soleus, gastrocnemius, and tibialis anterior muscles) | Mice were compared at 8 to 10 weeks of age. | ↑ Glycogen content (both transgenic models; EDL, gastrocnemius, and tibialis anterior) ↑ GS activity (both transgenic models in presence of G6P; gastrocnemius) ↓ GP activity (wild-type AMPKγ3 mice; gastrocnemius) | Mechanism not described in detail, however, postulated that AMPKγ could alter the function of the AMPKβ subunit's glycogen-binding domain in stimulating the activity of the glycogen debranching enzyme. | 39 |
| | Wild-type and AMPK overexpressing mice (examined EDL, soleus, quadriceps, gastrocnemius, and tibialis anterior muscles) | Mice were compared at 9 to 12 weeks of age. | ↑ Glycogen content (quadriceps, gastrocnemius, and tibialis anterior) ↑ GS activity (gastrocnemius) | With AMPKγ overexpression, there was reduction of G6P content which may result from increased G6P utilization and glycogen synthesis. | 40 |
| SKIP repression | Mouse C2C12 muscle cells | Transfection of cultured C2C12 cells with siRNA against SKIP gene for 48 h. Insulin (10 and 100 nM) for 30 min. | ↑ insulin-stimulated glycogen synthesis and dephosphorylation of GS at Ser641 | SKIP negatively regulates the insulin-signaling pathway. Repression of SKIP allows for insulin-stimulated glycogen synthesis. | 49 |
| Oxidant Stress | Female lean Zucker rats at 9-10 weeks of age (examined soleus muscle) | Isolated muscle incubated for 2 h in the absence or presence of 100 mM glucose oxidase without or with 5 mM/ML insulin | ↑ basal glycogen synthesis and GS activity | Oxidant stress increased phosphorylation of insulin receptor, Akt at Ser473, and GSK-3β at Ser9. | 50 |
| | Male ddY mice (examined gastrocnemius muscle) | Mice fed 1 of 3 test diets for 5 weeks using AIN-93 protocol: control diet, control+39.550 g/kg of whey amino acids, control+50.00 g/kg of whey protein hydrolysates. | ↑ Glycogen content (whey protein hydrolysates>whey amino acids>control) ↑ GS mRNA expression and GS protein level (whey protein hydrolysates) ↓ GS phosphorylation at Ser641 (whey protein hydrolysates) | Consumption of Whey protein decreases GS phosphorylation at Ser641 and could increase 6-phosphofructokinase (regulatory glycolytic enzyme) activity to increase GS and glycogen content. | 52 |
| | Rat L6 skeletal muscle cells | Differentiating myotubes pre-treated for 16 h with 1 mM linoleate in the absence or presence of insulin (100 nM) for 1 h. | ↑ basal and insulin-stimulated GS protein level (insoluble fraction) | GS was sequestered from activation by insulin treatment due to lipid treatment. | 53 |
| | Control and streptozotocin-induced diabetic male Sprague-Dawley rats (examined hindquarter muscle) | Rats were treated with insulin (4 IU/kg), OA (80 mg/kg), or the combination of OA+insulin in acute (60 min) and sub-chronic (14 days) studies. | ↑ GS activity after insulin+OA in diabetic group | OA enhances insulin signaling pathway including phosphorylation of Akt at Ser473 | 56 |
that correlated to decreases in GS phosphorylation [29]. It has been shown that exercise-induced activation of muscle GS in obesity and Type 2 Diabetes Mellitus (T2DM) is associated with dephosphorylation of GS at sites Ser7, Ser10, Ser640, and Ser644 [30]. These results were further confirmed in another study using obese individuals displaying normal glucose tolerance and T2DM subjects with their GS fractional activity (GS activity in the presence of G6P divided by total GS activity) being increased and GS phosphorylation decreased post-exercise [31]. In obese individuals with T2DM and obese non-diabetic individuals, a 10-week endurance exercise regime increased protein content of Akt, Cdc16 domain family, member 4 (TCBD14), S6-AMP-Activated Protein Kinase (AMPK), GS, and GLUT4 in their vastus lateralis muscle [32]. Furthermore, in 12 elderly male subjects who completed 8 week exercise training, GS and glycogen content were increased in their vastus lateralis muscle [33]. The dephosphorylation of GS at Ser7/10/640 was enhanced after the exercise intervention [33]. Another group who investigated the effects of endurance training on glucose metabolism in skeletal muscle found that in middle-aged males who regularly perform endurance physical activity had significantly higher GS protein levels than that of healthy sedentary controls [34]. Post-menopausal obese women with impaired glucose tolerance, who performed either a 6-month period of caloric restriction (subtract 500 calories daily from their typical diet) or caloric restriction+aerobic exercise (3x/week for 45 min), those who performed both exercise and caloric restriction had greater GS activity than those with only the caloric restriction [35]. Collectively, these results indicate that repetitive muscular contractions may be beneficial in obese, insulin-resistant, and elderly individuals in regards to the regulation of glucose.

To understand the mechanisms for exercise-induced increases in muscle glycogen, the effects of exercise training on glycogen regulatory proteins in female Sprague Dawley rats performing voluntary wheel running for 1, 4 or 7 weeks were investigated [36]. It was found that rat muscle glycogen and GS did not change after 1 week of training, but increased significantly after 4 and 7 weeks. Consistent with the GS activity, one of its primary activators, PP1 activity was also significantly increased after 4 and 7 weeks of training [36]. Exercise is also associated with a phenomenon known as glycogen overcompensation which prepares the body for bouts of strenuous exercise. When the tibialis anterior of rabbits were stimulated for 24 h to mimic exercise, glycogen level increased and remained at least 50% greater than the basal level for 6 h after stimulation [37]. The increase in glycogen correlated with a decrease in GP activity and an increase in the GS activity due to its dephosphorylation [37].

Components in signal transduction pathways

Mice with the muscle-specific knockout of GLUT4 have been shown to have increased GS activity and decreased GP activity following an overnight fasting (basal state), whereas contraction-induced glycogen breakdown remains normal [38]. The GLUT4 knockout mice display reductions in glucose transport that are associated with reductions in the IRS-1/Akt-PI3K signaling pathway. However, the increases in GS during basal state in these mice are correlated to increases in the activity of PP1 and protein levels of hexokinase II, muscle-specific regulatory subunit (R\(_{\text{mS}}\)), and Protein Targeting to Glycogen (PTG) compared to wild-type mice [38]. Therefore, the catalytic activity of PP1 of mice in fasting state seems to dominate over the actions of GSK-3 effects, allowing for the increase in muscle glycogen content.

Of the AMPK heteromeric complex, the \(\gamma\) subunit is specifically expressed in skeletal muscle [39]. Transgenic mice with skeletal muscle-specific overexpressing the y3 AMPK subunit had increased glycogen content and GS activity, and decreased GP activity [39]. Another group who developed a murine model of chronic skeletal muscle AMPK activation, found a selective increase in skeletal muscle glycogen content [40]. This increase in glycogen content was attributed to the catalytic a-subunit of AMPK [40].

A study aimed to elucidate the role of the GSK-3a in insulin resistance of human skeletal muscle cells from non-diabetic subjects found that the reduction of GSK-3a expression resulted in an increase of the insulin-induced glucose uptake and GS fractional velocity. However, the same effects were not witnessed in the basal state [41].

In a study investigating insulin-stimulated GS activity in human skeletal muscle, the increase in GS activity was positively associated with phosphorylation of Akt at Thr308 site and total Akt expression while both proteins were shown to be negatively associated with NH2-terminal GS phosphorylation [42]. The authors reported no association with the activation of GS with the phosphorylation of Akt at Ser473 site or with IRS-1-PI3K signaling [42]. In cultured human skeletal muscle cells, it was shown that the knockdown of GSK-3\(\beta\) using siRNA had no effect on total GS activity. However, GS phosphorylation was decreased due to the absence of GSK-3\(\beta\) leading to increase in GS fractional velocity at the basal as well as insulin-stimulated states [43].

Pompe disease, also known as Glycogen Storage Disease (GSD) type II, is caused by deficiency of lysosomal acid alpha-glucosidase (GAA). Muscle lysates collected from GAA–/– mouse strains had elevated GS, glycogenin, hexokinase, and G6P, and decreased GP activity in the absence of AMP [44]. It has been shown that recombinant human GAA (rhGAA) can improve the clinical outcomes of Pompe disease with variable results [45]. The co-administration of ramapycin (mTORC1 inhibitor) with rhGAA in a GAA knockout mouse model led to reduction of glycogen content in muscle more than rhGAA or ramapycin alone, suggesting inhibition of mTORC1 signaling can provide outcome benefits in certain GSDs [45].

Cytokines

Interleukin-6 (IL-6) is a cytokine that has both anti- and pro-inflammatory actions depending on the tissue affected [46]. A group who used primary human skeletal muscle cells to investigate the role of IL-6 in muscle cell differentiation and glucose metabolism found that chronic exposure of the cells to IL-6 (25 ng/mL) resulted in an increase in glucose uptake and incorporation into glycogen which was associated with increased gene expression levels of GLUT4 and GS.
The effect that IL-6 had on glucose metabolism was attributed to the regulation of the PI3-kinase pathway since the PI3-kinase inhibitor LY294002 suppressed the aforementioned IL-6 effects [47].

Another cytokine that has an impact on glucose metabolism and insulin resistance is adiponectin. Plasma adiponectin has been found to be significantly reduced in T2DM subjects compared to obese and lean subjects [48]. In these same subjects, plasma adiponectin level was positively associated with insulin-stimulated glucose disposal and oxidation, and stimulation of skeletal muscle GS activity, but negatively associated with lipid oxidation after adjustment for body fat [48].

A protein known as Skeletal muscle and Kidney-enriched Inositol Phosphatase (SKIP) is a phosphatase that converts P(1,3,4,5)P3 to P(1,3,4)P2, which negatively regulates the insulin-induced glycogen synthesis in skeletal muscle [49]. It has been shown that overexpression of the wild-type SKIP gene in differentiating mouse C2C12 skeletal muscle cells leads to decreases in the insulin-stimulated glycogen synthesis. However, using siRNA that targeted the SKIP gene allowed insulin to induce the phosphorylation of Akt and GSK-3β with the subsequent dephosphorylation and activation of GS [49]. Obviously, the SKIP gene could be of much research interest in discovering possible therapeutic interventions for GSDs.

The molecular actions of oxidant stress on insulin signaling and glucose transport have been studied in isolated soleus muscle of lean Zucker rats. It was found that oxidant stress (100 mU/mL glucose oxidase) alone led to significant increases in basal glucose transport, glycogen synthesis, and GS activity concomitant with increases in the phosphorylation of insulin receptor, Akt at Ser473, and GSK-3β at Ser9 [50]. However, oxidant stress had negative effects on the insulin-stimulated glucose transport and glycogen synthesis [50].

The effects of Transcription Factor E3 (TFE3) were examined in transgenic mice who selectively expressed TFE3 in their skeletal muscle to better understand its effects on skeletal muscle glucose regulation [51]. The glycogen content in skeletal muscle of TFE3 transgenic mice was more than twofold higher than that of wild-type mice, and this effect was associated with an upregulation of the mRNA expression levels of GLUT4, hexokinase II, and GS. Interestingly, these transgenic mice also displayed enhanced insulin sensitivity and exercise endurance capacity [51].

NUTRITIONAL FACTORS

When mice were fed an experimental diet containing Whey Protein Hydrolysates (WPH), a mixture of Whey Amino Acids (WAAs), or casein for 5 weeks, the gastrocnemius muscle glycogen level was significantly higher in the WPH group than in the WAA or casein group [52]. Furthermore, the WPH group exhibited significantly higher total GS protein level and lower phosphorylated GS level than the WAA or control group had [52].

A study using rat L6 skeletal muscle cells found that linoleate (1 mM) can increase GS protein in insoluble, but not cytosolic or membrane fractions during both the basal and insulin-stimulated state [53]. Studies have shown that the plant derived Oleanolic Acid (OA) can have antidiabetic effects [54,55]. One study investigated the effects of OA in skeletal muscle of streptozotocin-induced diabetic male Sprague Dawley rats [56]. Following treatment with insulin (4 IU/kg), OA (80 mg/kg), or the combination of OA-insulin for 14 days, GS activity was significantly increased in diabetic rats receiving both OA and insulin compared with non-diabetic and diabetic controls [56].

PHARMACEUTICAL REAGENTS

Women with Polycystic Ovary Syndrome (PCOS) typically display reduced insulin-mediated glycogen synthesis in skeletal muscle [57]. After a 16-week treatment with pioglitazone (30 mg/day), patients with PCOS displayed improved insulin-stimulated glucose metabolism and GS activity. This action was attributed to restoration of insulin’s ability to dephosphorylate GS [57].

FACTORS ABROGATING GS REGULATION

Studies performed in the past decade have elucidated a number of factors that regulate GS negatively in both human (Table 3) and animal (Table 4) model systems.

GENETIC DEFECTS

It has been reported that a frameshift mutation in PPP1R3A, the gene encoding RGL, resulted in decreased skeletal muscle GS activity with concomitant increases in GP activity in mice [58]. Humans expressing this variant have low basal and postprandial muscle glycogen content [58]. Pompe disease is a rare condition characterized by abnormal glycogen regulation leading to myopathy [59,60]. In an effort to find ways to decrease skeletal muscle glycogen accumulation seen with Pompe disease, a group of researchers discovered that a Phosphorodiamidate Morpholino Oligonucleotide (PMO) designed to invoke exon skipping in the transcript for muscle specific GS led to a dose-dependent decrease in GS transcripts in the quadriceps of mice [61]. This resulted in the reductions of skeletal muscle GS level and activity in the mice [61].

COMPONENTS IN SIGNAL TRANSDUCTION PATHWAYS

Regulation of AMPK pathway: In isolated rat muscle treated for 40 min with the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside (AICAR, 2 mM) and insulin (1 μM), insulin-stimulated GS activity was decreased whereas lactate release was markedly increased [62]. Similar results were seen in another study in which rat soleus muscle was treated for 30 min with AICAR (2 mM) before insulin (100 nM) was added to the samples for 45, 60 and 75 min [63]. AICAR treatment significantly suppressed insulin-stimulated glycogen synthesis which correlated with the increase in the phosphorylation of GS [63]. The AICAR treatment in this study also led to a significant increase in lactate production in rat soleus muscles [63].

Another study investigated the mechanism by which AMPK activation regulates muscle glycogen levels used AICAR, insulin, and adrenaline treatments on the Extensor Digitorum Longus (EDL) muscle of C57BL/6 mice [64]. After the treatment for 40 min, AICAR (2 mM) resulted in a modest but significant decrease in GS activity while no change in GS or GP phosphorylation was detected. However, treatment with adrenaline (10 μM) led to a strong increase in phosphorylation of GS at Ser9 and Ser11, and in phosphorylation of GP at Ser15 which correlated with a decrease in GS activity [64]. This same study measured the effect of AICAR on GS activity in EDL muscle isolated from transgenic mice with mutant AMPKα2 (kinase dead, K45R mutation) or wild-type mice. In unstimulated muscle, the GS activity in AMPKα2 mutant mice was significantly higher than that in wild-type littermates. This was correlated with a decrease in phosphorylation of GS at sites Ser9 and Ser641. Furthermore, AICAR treatment failed to reduce GS activity in AMPKα2 mutant mice whereas wild-type mice had a significant decrease [64]. It has also been shown in primary human muscle cell cultures that a 5 h insulin treatment (300
| Factor/Manipulation | Participants/Model System | Treatment/Manipulation; Duration | Findings | Proposed Mechanisms | Citation |
|---------------------|---------------------------|---------------------------------|----------|---------------------|----------|
| Pro-longed insulin treatment | Human skeletal muscle cells | Insulin (300 nM) - 5 h | ↑ Glycogen content ↓ GS activity and glycogen synthesis rate | Pro-longed insulin treatment could result in a negative feedback mechanism on insulin signaling pathway | 65 |
| Adrenaline | Male and female healthy subjects (examined tibialis anterior muscle) | Adrenaline (0.05 µg/kg/min) or saline infusion for 240 min. Euglycaemic-hyperinsulinemic clamp for 120-240 min | ↓ insulin-stimulated Glycogen synthesis, GS activity, and dephosphorylation of GS at Ser641 | Adrenaline blocks insulin-mediated GS activation most likely due to preventing dephosphorylation of GS at Ser641. | 66 |
| T2DM | Obese subjects with or without T2DM, and lean non-diabetic subjects (examined vastus lateralis muscle) | Following overnight fast, lean and obese controls underwent a euglycemic-hyperinsulinemic clamp (4 h) with tracer glucose and combined with indirect calorimetry; diabetic patients were clamped twice with either a euglycemic or an isoglycemic-hyperinsulinemic clamp (fixing glucose at the fasting glucose level) separated by 4-6 weeks | ↓ insulin-stimulated GS activity during euglycemia ↓ insulin-stimulated dephosphorylation of GS at Ser7/10 during euglycemia and hyperglycemia | T2DM impairs insulin-stimulated glycerogen synthesis involving Akt phosphorylation at Ser473 and Thr308, and dephosphorylation of GS at Ser7/10. | 69 |
| PCOS | Obese women with or without PCOS (examined vastus lateralis muscle) | Euglycemic-hyperinsulinemic clamp performed for 3 h. | ↓ insulin-stimulated GS activity and dephosphorylation of GS at Ser7/10 (PCOS vs. control) | PCOS decreased the insulin-mediated dephosphorylation of GS at Ser7/10. | 57 |
| Physical Inactivity | Healthy male subjects (examined vastus lateralis muscle) | Bed rest (all transport of subjects took place in wheelchairs) - 7 days | ↓ GS activity ↓ GS phosphorylation at Ser7/10 | Physical inactivity/bed rest impairs muscle insulin signaling and dephosphorylation of GS at Ser7/10 | 71 |
| High glucose+angiotensin | Human skeletal muscle cells | Muscle cells were incubated with or without 25 mM glucose for 24 h. Angiotensin treatment (1 µM) - 20 min | ↓ GS activity ↓ GS phosphorylation at Ser640/641 | Angiotensin reduced ERK1/2 phosphorylation at Thr202/Tyr204 and impaired dephosphorylation of GS at Ser640/641. | 72 |

**Abbreviations:** BMI=Body Mass Index, ERK=Extracellular Regulated signal Kinase, GS=Glycogen Synthase, GSK=Glycogen Synthase Kinase, T2DM=type 2 Diabetes Mellitus, ↑=significant increases due to factor/manipulation, ↓=significant decreases due to factor/manipulation.

| Factor/Manipulation | Participants/Model System | Treatment/Manipulation; Duration | Findings | Proposed Mechanisms | Citation |
|---------------------|---------------------------|---------------------------------|----------|---------------------|----------|
| PPP1R3A Mutation | Wild-type and mutant (premature stop mutation in PPP1R3A) mice Parent strain - Chimeric mice cross-bed with C57BL/6J mice (examined muscle not specified) | Tissues collected during ad libitum. Age used not described. | ↓ Glycogen content ↑ GS activity ↑ GP activity | The reduction in GS activity was most likely due to the inability of PP1 to colocalize with GS and to bind to glycogen. | 58 |
| GS-PMO | C57BL/6+GAA-/+ mice (examined quadriceps muscle) | GS-PMO administered at 15 or 30 mg/kg bodyweight by tail vein injection once every 2 weeks for a total of 12 weeks, muscle isolated 2 weeks after stopping treatment | ↓ Glycogen content, GS activity, GS mRNA expression, and GS protein level | GS-PMO forces exon skipping in the processing of Gys1 mRNA, leading to a premature termination codon. | 61 |
| AICAR | Male Sprague-Dawley rats (examined epitrochlearis muscles) | AICAR (2 mM) and insulin (1 µM) - 40 min | ↓ basal and insulin-stimulated GS activity | AICAR treatment favors an increase in glycolytic flux instead of glycogen synthesis. | 62 |
| | Male Albino-Wistar rats (examined soleus and epitrochlearis muscles) | AICAR (2 mM) and insulin (100 nM) - 45, 60, and 75 min (preincubated with AICAR 30 min before insulin added) | ↓ insulin-stimulated glycogen synthesis and glycogen content (soleus muscle) ↓ GS phosphorylation at Ser641 (45 min, soleus muscle) | AICAR treatment led to a time-dependent reduction in Akt phosphorylation at Thr308 and activation of GSK-3α/β. | 63 |

**Table 3:** Factors negatively impacting GS regulation in studies using human materials.

**Table 4:** Factors negatively impacting GS regulation in animal studies.
Adrenaline

C57BL/6 J mice (examined EDL muscle)  
Adrenaline (10 µM) - 40 min  
↓ GS activity  
↓ GS phosphorylation at Ser8/11, GP activity, and GP phosphorylation at Ser15  
Adrenaline impairs dephosphorylation of GS at Ser8/11 while promoting GP phosphorylation at Ser15.  
64

Male Wistar rats (examined soleus muscle)  
Adrenaline (10 µM) - 30 min  
↓ GS activity  
(Ser645/649/653/657) phosphorylation  
Adrenaline impairs dephosphorylation of GS at Ser645/649/653/657 most likely through PKA signaling pathway, which may also lead to decreases in PP1 activity.  
67

Wild-type and null GM allele (deletion encompassing 1st exon encoding PP1) mice  
Parent strain: C57BL/6 mice (examined hind limb muscles)  
Adrenaline (0.5 mg/g) for 15 min, insulin (150 mU/g) for 20 min or saline intraperitoneal injections  
↓ GS activity  
↓ GP activity  
Adrenaline’s effects may be attributed to decrease in PP1 activity and/or increase in phosphorylase kinase activity.  
68

Age

Male Fischer 344 rats (examined soleus and tibialis anterior muscles)  
Old (24 months) rats compared to young (6 months) rats maintained ad libitum or calorie restricted  
↓ GS and GP activity and GS protein levels (soleus)  
↓ GS phosphorylation at Ser640 (soleus)  
Aging negatively affects GS and GP activity in soleus muscle, which may be associated with impairment of dephosphorylation of GS at Ser640.  
70

Oxidant Stress

Female lean Zucker rats (examined soleus muscle)  
Isolated muscle from 9-10 week old rats incubated for 2 h in the presence of 100 µM/mL glucose oxidase, without or with 5 µM/mL insulin.  
↓ insulin-stimulated GS activity and glycogen synthesis  
Oxidant stress was found to be associated with decreased phosphorylation of insulin receptor, Akt at Ser473, and GSK-3β at Ser9.  
50

GM null allele

Wild-type and null GM allele (deletion encompassing 1st exon encoding PP1) mice  
Parent strain - C57BL/6 mice (examined hind limb muscles)  
Adrenaline (0.5 mg/g) for 15 min, insulin (150 mU/g) for 20 min, or saline intraperitoneal injection  
↓ insulin-stimulated GS activity (null GM mice)  
↓ GS phosphorylation at Ser7/640/644 and GP phosphorylation at Ser14 (null GM mice)  
Decreased PP1 activity allows for GS and GP phosphorylation which may also be attributed to increases in phosphorylase kinase activity.  
68

Dexamethasone

Male Sprague-Dawley rats (examined gastrocnemius muscle)  
4 groups: saline, 10% sucrose drinking solution, dexamethasone (1 mg), and dexamethasone+sucrose - 7 days.  
↓ Glycogen content (dexamethasone alone)  
↓ GS activity (dexamethasone and dexamethasone+sucrose)  
Dexamethasone may regulate GSK-3β and/or CAMKII activity.  
75

Abbreviations: AICAR=5-Aminomimidazole-4-Carboxamide-1-b-D-Ribonucleotide, AMPK=5’-AMP-Activated Protein Kinase, CAMKII=Calcmodulin Kinase II, EDL=Extensor Digitorum Longus, GP=Glycogen Phosphorylase, GS=Glycogen Synthase, mRNA=messenger Ribonucleic Acid, PKA=Protein Kinase A, PMO=Phosphorodiamidate Morpholino Oligonucleotide, PP1=Protein Phosphatase 1. ↑=significant increases due to factor/manipulation, ↓=significant decreases due to factor/manipulation.

In the presence of adrenaline, the older rats (24 months) showed lower GS and GP activity compared to young rats, which may be associated with decreased phosphorylation of GS at Ser640 and increased phosphorylation of GP at Ser15 [65]. However, treatment with AICAR (2 mM) restored the glycogen synthesis and GS activation in the 5 h insulin-treated cells [65].

The major skeletal muscle glycogen targeting subunit (GM) of PP1 has also been investigated in regards to control by various hormones [68]. GM−/− mice had increased phosphorylation of GP at Ser7 and GS at Ser7, Ser640, and Ser644 along with a decrease in insulin-stimulated GS activity compared to GM+/+ mice [68]. This indicates that the GM subunit of PP1 complex is the major phosphatase complex that leads to the dephosphorylation of these sites in vivo. Adrenaline (0.5 mg/g) treatment for only 15 min led to a significant increase in GP activity while decreasing GS activity in control mice [68]. However, the same treatment conditions yielded no significant changes in GP or GS activity in GM−/− mice, suggesting that the GM subunit of PP1 is required for adrenaline’s actions [68].

Akt pathway: Individuals with T2DM display lower GS activity and increased GS phosphorylation during the recovery period post-exercise, which implies these individuals, have impaired response to exercise [30]. Compared to non-diabetic controls, patients with T2DM undergoing a euglycemic-hyperinsulinemic clamp displayed an impairment of the insulin-stimulated glucose disposal and glucose oxidation during euglycemia [69]. This effect was associated with impairment of insulin-stimulated GS activity and decrease of GS dephosphorylation with reduction of Akt phosphorylation at Thr308 and Ser473. During hyperglycemia, the insulin-stimulated glucose disposal and oxidation normalized, however, the dephosphorylation of GS as well as phosphorylation of Akt at Thr308 and Ser473 remained impaired [69]. Women with PCOS have the risk of developing insulin resistance in skeletal muscle. Compared to women without PCOS, women with PCOS display the reduction of insulin-stimulated GS activity and dephosphorylation of GS [57].

Aging

Aging also seems to play a role in altering GS level and glycogen accumulation. It has been shown that the older rats (24 months) displayed reduced GS and GP activity, lowered muscle GS protein levels, and increased phosphorylation of GS in soleus muscle than young mice (6 months) [70]. Along with aging, extended bed rest occurs. One group who investigated the mechanisms behind physical inactivity-induced insulin resistance in the skeletal muscle (vastus lateralis) of 12 healthy male subjects after 7 days of bed rest found that the ability of insulin to activate GS was reduced [71]. They found that phosphorylation of GS at Ser7 and Ser10 increased following bed rest [71]. Interestingly, in rat
soleus muscle during the basal state, oxidant stress (100 mU/mL glucose oxidase) increased GS activity. However, GSK-3β remained active in the presence of both oxidant stress and insulin [50]. The presence of oxidant stress also significantly inhibits the insulin-induced glucose transport and glycogen synthesis [50].

Hormones

In human muscle cells treated with or without 25 mM glucose for 24 h in combination with angiotensin (1 µM), glucose deprivation plus angiotensin increased levels of ERK½ phosphorylation and GSK-3β (Ser9) phosphorylation [72]. However, only those treated with high glucose and angiotensin displayed an increase in GS phosphorylation at Ser640/641 which correlated with a decrease in GS activity [72].

Glucocorticoids such as dexamethasone have been known to have an impact on glucose and fat metabolism, which could lead to alterations such as insulin resistance and dyslipidemia [73,74]. In the red and white gastrocnemius muscle of rats, the administration of dexamethasone (1 mg/rat/day) increased glycogen concentration while reducing GS activity in both muscle fiber types [75]. Rat treated with dexamethasone and sucrose had decreased muscle glycogen content compared with the dexamethasone alone group. However, this effect was also associated with a decrease in GS activity similar to that seen in animals treated with the dexamethasone alone [75].

Perspectives

Although the glycogen synthesis pathway has been studied extensively, there seems to be more to be investigated. For example, GSK-3 is known as a major regulator of GS activation through phosphorylation. Using GSK-3 knock-in mice with GSK-3α and -β genes being replaced with mutant forms (GSK-3αβ/β/β), it has been shown that the insulin-mediated inhibition of GSK-3 is not a rate-limiting step in skeletal muscle glycogen synthesis [76]. Even though insulin failed to promote the activation of GS in these GSK-3α/β mutant mice, the glycogen content within the skeletal muscle of these mice were similar to their wild-type controls [76].

One determinant of GS activity is the existing amount of glycogen within the muscle as well as muscle fiber type [77,78]. As demonstrated in rodents, low glycogen content was associated with decrease in GS phosphorylation and high glycogen content was associated with decrease in GS activation [79-81]. One group has shown that epinephrine stimulates glycogenolysis in Wistar rat skeletal muscles with normal and high, but not low, glycogen content. The glycogenolysis actually being associated with decreases in GS phosphorylation and increases in GS activity [82]. This demonstrates an association between GS activation and glycogen content in this condition.

Physical activity has also been shown to affect glycogen content in rodents with the phosphorylation of GS being reduced 30 min post-exercise, but returning to baseline at 90 min post-exercise [83]. As described previously, physical activity can result in the enhancement of GS activity. On the other hand, it is also well known that during vigorous physical activity, endurance or strength training, the skeletal muscle relies a great deal on the breakdown of glycogen, as well as fat, to provide adequate energy [84]. Besides depletion of muscle glycogen stores, skeletal muscle protein degradation will also occur during such physical activity [85]. The depletion of glycogen will result in the increase of proteolysis to get amino acids and β-oxidation of fatty acid from muscle fat and reduction of pyruvate oxidation [86]. Therefore, nutritional strategies should be implemented during the post-recovery period to maximize skeletal muscle glycogen and protein synthesis. Besides for the importance of replenishing endogenous substrate stores, optimal nutritional intake can assist in facilitating the repair of damaged muscle [87]. For athletes, nutrition can play a key role in determining the adequate period of time between competitions for complete muscle recovery. Similarly, pre-physical activity nutrition can have a major impact on metabolism and activity during performance [88]. Further research is needed to optimize pre- and post-physical activity nutritional strategies involving the investigation of the regulation of key enzymes in glycogenesis such as GS, GP, and GSK-3.

From a pharmaceutical standpoint, having a greater understanding of the regulation of glycogen synthesis could be beneficial to a number of individuals suffering from a variety of diseases. With the millions of insulin-resistant men and women, the development of glycogen regulating enzyme activators/inhibitors could help alleviate the formation of other conditions such as myopathy. GP inhibitors, for instance, have been receiving attention for being possible therapeutic agents for diabetes and cancer [89]. The regulation of GSK-3 is also a topic of interest among many due to GSK-3 regulation being connected to a number of conditions such Alzheimer’s disease, cancer, diabetes, and general muscle wasting [90,91]. Therefore, GSK-3 inhibition may provide therapeutic effects to a broad range of conditions.

Conclusion

The purpose of this review has been to compile relevant research related to the regulation of GS over the past 10 years. As one can tell, there is much still being discovered regarding glucose metabolism in skeletal muscle. More in-depth studies performed both in vitro and in vivo are needed to grasp a full understanding of skeletal muscle GS regulation. With a greater understanding, possible therapeutic benefits could arise for individuals suffering from a variety of metabolic diseases.

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