*Original Article*

# Glucose Metabolism in Mice Lacking Muscle Glycogen Synthase

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**Glycogen is an important component of whole-body glucose metabolism. MGSKO mice lack skeletal muscle glycogen due to disruption of the *GYS1* gene, which encodes muscle glycogen synthase. MGSKO mice were 5–10% smaller than wild-type littermates with less body fat. They have more oxidative muscle fibers and, based on the activation state of AMP-activated protein kinase, more capacity to oxidize fatty acids. Blood glucose in fed and fasted MGSKO mice was comparable to wild-type littermates. Serum insulin was lower in fed but not in fasted MGSKO animals. In a glucose tolerance test, MGSKO mice disposed of glucose more effectively than wild-type animals and had a more sustained elevation of serum insulin. This result was not explained by increased conversion to serum lactate or by enhanced storage of glucose in the liver. However, glucose infusion rate in a euglycemic-hyperinsulinemic clamp was normal in MGSKO mice despite diminished muscle glucose uptake. During the clamp, MGSKO animals accumulated significantly higher levels of liver glycogen as compared with wild-type littermates. Although disruption of the *GYS1* gene negatively affects muscle glucose uptake, overall glucose tolerance is actually improved, possibly because of a role for *GYS1* in tissues other than muscle. *Diabetes* 54:3466–3473, 2005**

After a meal, glucose is distributed into various tissues of the body where it can be utilized as an energy source or stored as glycogen (1). Glycogen is a branched polymer of glucose residues connected by α-1,4-glycosidic linkages formed by the enzyme glycogen synthase (EC 2.4.1.11) and branch-points formed via α-1,6-glycosidic linkages, introduced by the branching enzyme (EC 2.4.1.18). There are two mammalian isoforms of glycogen synthase. One, encoded by the *GYS2* gene, appears to be expressed only in liver (2) while a second gene, *GYS1*, is expressed in skeletal and cardiac muscle as well as adipose tissue, kidney, and brain (3).

Estimates of the contribution of skeletal muscle glycogen to glucose disposal after ingestion of carbohydrate vary. In humans, reports of ingested glucose conversion to muscle glycogen range from ~40% (4) up to 90% (5). It is widely accepted that muscle is an important site for glucose disposal and one might hypothesize that, in the absence of muscle glycogen, glucose clearance would be impaired. Consistent with this hypothesis, mutations in the *GYS1* gene in humans have been implicated in certain diabetic populations with, for example, the Pro442Ala mutation resulting in decreased muscle glycogen synthase activity (6).

We recently described the MGSKO mouse, in which the *GYS1* gene is disrupted (7). Analysis of MGSKO mice confirmed the long-held supposition that glycogen synthase is required for glycogen synthesis since these animals were devoid of glycogen in cardiac and skeletal muscle (7). In the present study, we analyzed a number of metabolic parameters in the MGSKO mouse, including whole-body glucose metabolism, with an initial hypothesis that mice lacking the ability to synthesize muscle glycogen would be impaired in their ability to remove glucose from the circulation. We report that, despite impaired glucose uptake into skeletal muscle, glucose disposal is either normal or improved in MGSKO mice, depending on experimental conditions, and that lack of glycogen does not result in increased fat mass.

## RESEARCH DESIGN AND METHODS

**Generation of MGSKO mice.** Lexicon Genetics (The Woodlands, TX), utilizing their OmniBank library of gene-trapped mouse embryonic stem cell clones, generated *GYS1* heterozygous mice. Briefly, embryonic stem cells (129/SvEvBrd) were infected with the VICTR25 retroviral gene-trap vector, which integrated upstream of exon 12 of the GYS1 gene. Embryonic stem cells with normal karyotype were injected into C57/BL/6J blastocysts and implanted into pseudopregnant female mice. Pups were crossed with C57/BL/6J animals and gene disruption was confirmed by Southern Analysis. We mated these animals to generate *GYS1*-null mice (75% C57), denoted MGSKO mice, as previously described (7). Mice were maintained in temperature and humidity-controlled conditions with a 12-h light 12-h dark cycle and were allowed food and water ad libitum in the American Association for the Accreditation of Laboratory Animal Care–approved facility at Indiana University. All procedures were conducted according to National Institutes of Health guidelines and approved by the Indiana University School of Medicine Animal Care and Use Committee.

**High-fat feeding.** Mice at 40 days of age were placed on a high-fat diet for 16 weeks. Body weight and blood levels of triglyceride, glucose, and lactate were monitored on a weekly or biweekly basis. The high-fat diet (cat. no. F3282; Bio-Serv, Frenchtown, NJ) was 16% protein, 59% fat, and 25% carbohydrate with 5.286-kcal/g caloric equivalents. The fatty acid composition was 161 g/kg oleic, 89 g/kg palmitic, 44 g/kg stearic, 35 g/kg linoleic, 11 g/kg cis-9-hexadecanoic, 5 g/kg myristic, and 4.7 g/kg cis-11-eicosenoic, with all others at ≤1.5 g/kg. The caloric profile of the standard diet (Teklad cat. no. 7017; Harlan, Indianapolis, IN) was 22% protein, 11% fat, and 60% carbohydrate with 3.41 kcal/g metabolizable energy.

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ACC, acetyl-CoA carboxylase; AMPK, AMP-kinase; GTT, glucose tolerance test.

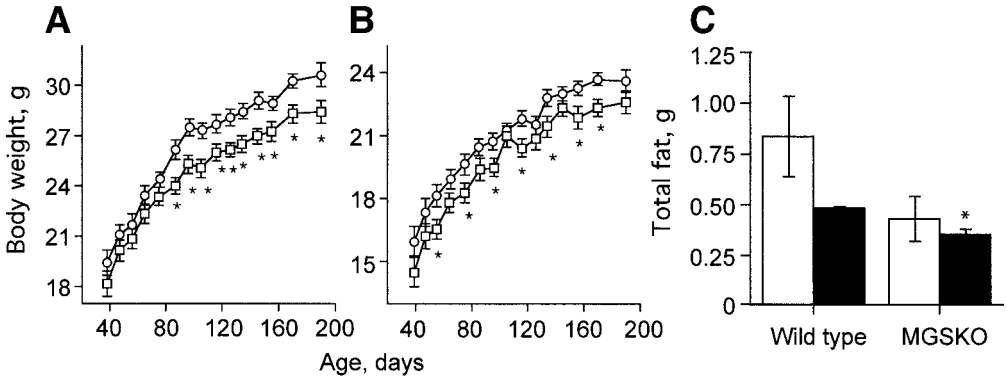


**FIG. 1. Growth rate and body composition. Male (*A*) and female (*B*) wild-type (○) and MGSKO (□) mice were weighed during growth. *n* = 11–29 (females), *n* = 18–37 (males). *C*: Whole-body fat content (□), *n* = 8, and epididymal fat mass (■), *n* = 5, **P* < 0.05 compared with wild type.**

**Preparation of samples for biochemical analyses.** Mice were killed by cervical dislocation or decapitation. Muscle and liver were rapidly excised, quick-frozen in liquid nitrogen, and stored at −80°C. Frozen tissue was powdered, and samples were homogenized as described previously (8). Glycogen synthase (9) was assessed in homogenates by monitoring the incorporation of glucose from UDP-[U-$^{14}$C]glucose into glycogen as previously described (7). Glycogen content in tissues was determined by measuring amyloglucosidase-released glucose from glycogen as previously described by Suzuki et al. (8).

**Western blot analysis.** Tissue homogenates were subjected to SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Millipore, Bedford, MA) that were then incubated with the antibodies to AMP-activated protein kinase (Cell Signaling Technology, Beverly, MA), phospho-AMP-kinase (AMPK) (Cell Signaling Technology), or phospho-acetyl-CoA carboxylase (Cell Signaling Technology). The secondary antibody was a rabbit anti-mouse/horseradish peroxidase (Sigma, St. Louis, MO). Binding of the antibody was detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ). Levels of protein expression were quantitated by densitometric scanning of autoradiograms.

**Muscle sectioning and staining.** The gastrocnemius of both MGSKO and control mice was embedded in a 3:1 solution of 20% sucrose in 0.1 mmol/l Sorrenson's buffer:OCT Polyfreeze Media (Polysciences, Warrington, PA). Frozen muscle tissue was cryosectioned at −20°C, and three sections were sequentially thaw-mounted on SuperFrost-Plus slides (Fisher Scientific, Hampton, NH). Muscle fiber typing by myosin ATPase staining (10) was performed on 12-μm-thick cryosections. A minimum of three sections per muscle were examined in duplicate and photographed by using a Nikon Microphot-FXA fluorescence microscope coupled to a charged-coupled device camera and image processing system (Scion Image 1.62c; National Institutes of Health). On all sections examined, at least three fields, comprising >100 different fibers, were randomly selected and examined.

**Glucose tolerance tests.** Mice were fasted overnight for 16 h followed by an intraperitoneal injection of glucose (2 mg/g body wt). Blood samples were collected from the tail at the indicated times, and blood glucose and lactate were measured with a DEX glucometer (Bayer, Mishiwaka, IN) and Lactate Pro (Arkray, Kyoto, Japan), respectively. Serum insulin levels were measured using the Sensitive Rat Insulin radioimmunoassay kit (Linco Research, St. Charles, MO) in blood samples (15 μl) taken from tail vein during the glucose tolerance test (GTT) as described (11).

**Euglycemic clamp.** Five-month-old conscious male mice, after fasting from 8 A.M. to 2 P.M., were subjected to the euglycemic-hyperinsulinemic technique, as described by Halseth et al. (12), at the Vanderbilt University School of Medicine Mouse Metabolic Phenotyping Center. Insulin was infused at a rate of 2.5 mU · kg$^{-1}$ · min$^{-1}$, and glucose infusion was regulated to maintain euglycemia. After 2 h, the glucose infusion rate was kept constant and a bolus of [2-$^{3}$H]deoxyglucose was given. Mice were killed 25 min after deoxyglucose administration, and vastus lateralis, soleus, gastrocnemius, diaphragm, heart, adipose tissue, and brain were excised and rapidly frozen in liquid nitrogen. Tissue-specific glucose uptake was determined in these tissues as described by Kraegen et al. (13).

**Body composition.** Subsequent to tissue removal for assessing tissue-specific glucose uptake, carcasses were lyophilized at the University of Cincinnati Medical Center Mouse Metabolic Mouse Phenotyping Center and subjected to petroleum ether extraction to determine fat, water, and lean body mass (14).

**Food consumption.** Food consumption was measured in 11- to 12-month-old male MGSKO and wild-type mice for 7 days. Preweighed food was given to mice at 5 P.M., and food remaining was determined the following afternoon (5 P.M.). The total amount of food consumed over the 7 days was used to calculate the average food consumption per day for each animal.

Blood ketones and triglycerides were monitored with a BioScanner 2000 (Polymer Technology Systems, Indianapolis, IN). Protein content was determined by the method of Bradford (15) using BSA as a standard. Results are presented as means ± SE. Unpaired Student's *t* test was used to determine statistical significance.

## RESULTS

Disruption of *GYS1*, encoding the muscle isoform of glycogen synthase, results in mice lacking glycogen synthase protein, activity, and glycogen in skeletal and cardiac muscle (7) as well as glycogen in lung, brain, and kidney (B.A.P., J.M.S., C.R. Cope, P.J.R., unpublished observations). A high percentage of MGSKO mice have abnormalities of cardiac development and die shortly after birth (7). Surviving null mice exhibited no obvious abnormalities (7). The survivors had normal locomotor activity and exercise endurance similar to wild-type mice (16).

**Growth and body composition.** There were no obvious differences, by visual inspection, in the size of MGSKO mice compared with wild-type littermates. However, when body weight was monitored carefully over time, it was apparent that, after ~3 months of age, male and female MGSKO mice were 10 and 5%, respectively, lighter than the wild-type animals (Fig. 1). The differences in weight did not appear to be related to food consumption since MGSKO and wild-type male mice ate similar amounts of

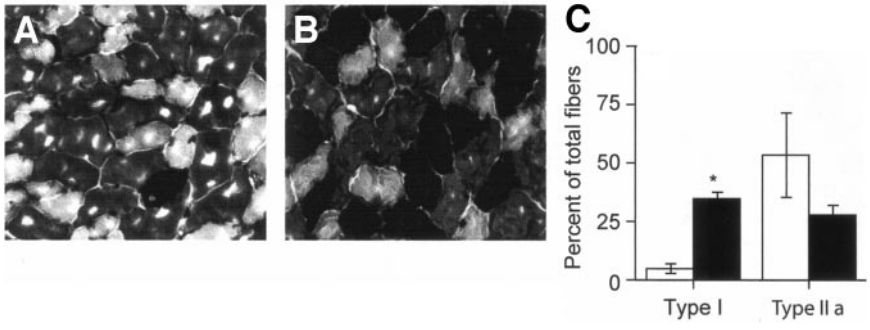


**FIG. 2. Fiber typing in skeletal muscle. Gastrocnemius muscles obtained from wild-type (*A*) and MGSKO (*B*) mice were stained for ATPase. Under conditions of the staining, type I fibers stain black, type IIA fibers stain pale, type IIB fibers stain intermediate. Representative pictures are shown. *C*: quantitation of type I and type IIA fibers. □, wild type; ■, MGSKO. **P* = 0.0004 compared with wild type**

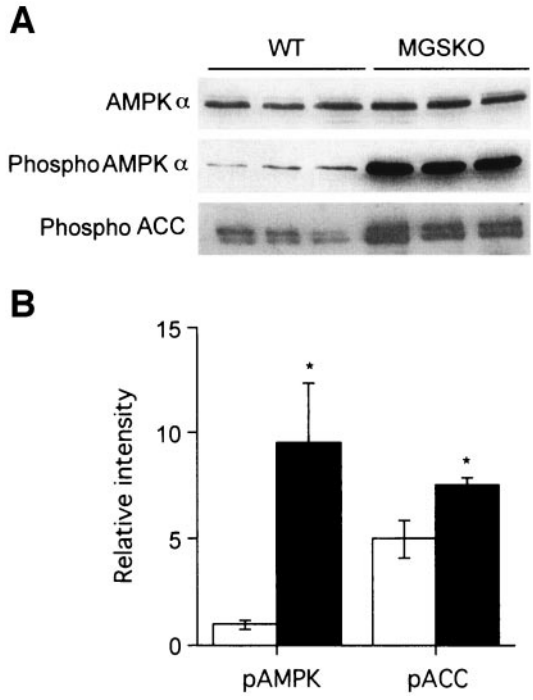


**FIG. 3. Phosphorylation state of AMPK and ACC in skeletal muscle. *A*: Whole hindlimb muscle from wild-type (WT) and MGSKO animals was analyzed by Western blotting for total AMPK, phosphorylated AMPK (pAMPK), and phosphorylated ACC (for pACC). *B*: Densitometric quantitation of phosphorylated AMPK and ACC. Wild-type values were normalized such that their average equals one (for pAMPK) or five (for pACC). *$P < 0.05$ compared with wild type.**

standard diet (0.130 ± 0.002 vs. 0.128 ± 0.005 g food · $day^{-1}$ · g body $wt^{-1}$, respectively).

One potential explanation for a decrease in body weight is a reduction in body fat. The mass of body fat, as determined from body composition analysis, showed a strong tendency to be reduced in male MGSKO mice (Fig. *1*). Consistent with this trend, epididymal fat pads from MGSKO-null mice weighed significantly less than those from wild-type littermates (Fig. *1*). Lean mass was similar between MGSKO animals and wild-type littermates (30.7 ± 0.5, wt; 31.0 ± 0.6% of carcass weight, MGSKO).

**Metabolism in MGSKO mice.** Upon necropsy, the muscle of MGSKO mice entered rigor mortis more rapidly than wild-type muscle and also had a redder appearance. The former observation is consistent with the absence of glycogen (17) and was also found in mice lacking the type 1 phosphatase glycogen-targeting subunit (8), which has a 90% reduction in muscle glycogen (A.A.D.R., unpublished observations). The increased redness would be consistent with a reduction in fat surrounding the muscle but could also be due to a higher proportion of oxidative fibers in the null mice. One method to assess the proportion of oxidative fibers is by ATPase staining (10). This analysis indicated that in the gastrocnemius muscle of MGSKO animals there was an increase in the number of slow, oxidative type I fibers compared with wild-type littermates (Fig. 2). The proportion of type II b oxidative glycolytic fibers was not significantly different, whereas the amount of fast glycolytic type II a fibers decreased, although the difference did not reach statistical significance. The capacity of a muscle for fatty acid oxidation can be indexed biochemically by the activation state of the AMPK and the phosphorylation state of its substrate acetyl-CoA carboxylase (ACC) (rev. in 18). Phosphorylation of ACC causes inactivation, leading to reduced malonyl CoA levels and thereby relieving inhibition of fatty acid oxidation (rev. in 19). The activation state of AMPK, as judged by phospho-specific antibodies to the activating T-loop phosphorylation site, was ninefold higher in the MGSKO mice than in wild type (Fig. 3). Consistent with this elevation, the phosphorylation state of ACC was increased by 52% (Fig. 3). To make sure that the activation of AMPK was not an artifact of tissue harvesting, with more AMP generation in muscle lacking glycogen, we also analyzed muscle taken from anesthetized mice by in situ freeze clamping. The samples were compared with muscle removed from the contralateral limb and then frozen in liquid nitrogen. The greater activation of AMPK in the MGSKO muscle was observed whether or not the tissue was freeze clamped (data not shown). These results suggest a greater basal capacity for fatty acid oxidation in the muscle of MGSKO mice.

One might hypothesize that elimination of a major repository for circulating glucose would affect their ability to regulate blood glucose levels. However, blood glucose levels in fed and fasted animals were normal in MGSKO mice as were the levels of triglycerides and ketones (Table 1). The lactate level in fed MGSKO mice was significantly lower but the difference was minimal after fasting (Table 1). In response to a glucose challenge, both male and female MGSKO mice cleared glucose from the bloodstream significantly better than either wild-type or heterozygous littermates during an intraperitoneal GTT (Fig. 4). The initial increase in blood glucose was similar up to 5 min, after which the levels in the MGSKO mice remained lower than in controls (Fig. 4, *inset*). To be sure that differences in GTT performance were not in some way linked to glucose absorption from the peritoneal cavity, we also performed GTT's with glucose administered orally. As with intraperitoneal delivery, the MGSKO mice disposed of the glucose more effectively (data not shown).

Because glucose could not be stored as glycogen in muscle, it is possible that more glucose was metabolized



TABLE 1
Blood metabolite levels of fed and overnight-fasted wild-type and MGSKO mice

| | Fed | | | | | Fasted | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wild type | | MGSKO | | | Wild type | | MGSKO | | |
| | Male | Female | Male | Female | *n* | Male | Female | Male | Female | *n* |
| Glucose (mg/dl) | 129 ± 6 | 118 ± 6 | 129 ± 7 | 112 ± 4 | 6 | 91 ± 7 | 78 ± 5 | 80 ± 9 | 72 ± 3 | 10 |
| Lactate (mmol/l) | 2.7 ± 0.2 | 2.9 ± 0.4 | 1.9 ± 0.1* | 1.6 ± 0.2* | 6 | 1.6 ± 0.1 | 1.9 ± 0.0 | 1.5 ± 0.1 | 1.6 ± 0.1* | 5 |
| Triglyceride (mg/dl) | 82 ± 10 | 76 ± 11 | 85 ± 10 | 88 ± 7 | 5–6 | ND | ND | ND | ND | |
| Ketone (mg/dl) | 4.4 ± 0.2 | 4.6 ± 0.4 | 4.2 ± 0.3 | 4.2 ± 0.1 | 5–7 | 18.6 ± 1.2 | 18.6 ± 1.4 | 18.1 ± 1.1 | 22.2 ± 2.3 | 9–13 |
| Serum insulin (ng/ml) | 1.8 ± 0.3 | ND | 0.8 ± 0.1* | ND | 13–20 | 0.41 ± 0.03 | ND | 0.49 ± 0.10 | ND | 5–7 |

Data are means ± SE. *$P < 0.05$ compared with same-sex wild-type littermate. ND, not determined

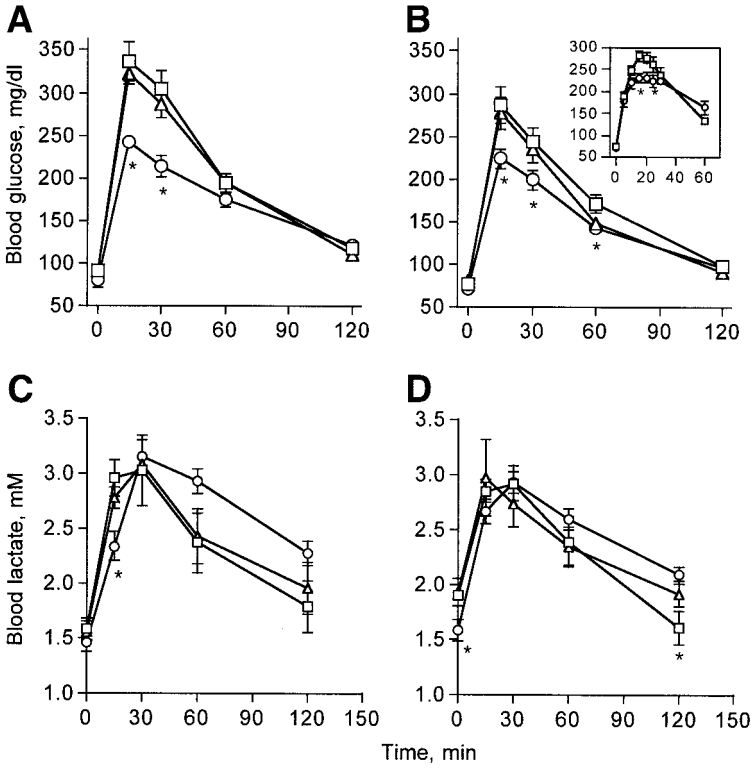


**FIG. 4. Glucose tolerance in mice on a standard diet. Five-month-old male (*A*, *C*) or female (*B*, *D*) wild-type (□), heterozygotic (△), and MGSKO (○) mice were subjected to a GTT and blood glucose (*A*, *B*) and lactate (*C*, *D*) levels were monitored. *Inset*: GTT with increased frequency of blood glucose sampling. Area under the curve calculations yielded 11.7 ± 0.8 wild type, 9.7 ± 0.7 heterozygous null, 9.1 ± 0.7 MGSKO arbitrary units ($P = 0.025$ for female wild type vs. MGSKO). For male mice, similar calculations yielded 13.9 ± 0.6 wild type, 13.3 ± 0.5 heterozygous null, 10.9 ± 1.1 MGSKO arbitrary units ($P = 0.03$ for wild type vs. MGSKO). $n = 10$, *$P < 0.05$ compared with wild type.**

anaerobically by the muscle to produce lactate in the MGSKO mice. Blood lactate levels during the GTT were not significantly different between genotypes, although in male MGSKO mice a delay in lactate production was observed as well as a trend to higher lactate levels at 60 and 120 min after glucose injection (Fig. 4). Heterozygous mice had blood lactate levels indistinguishable from wild-type littermates (Fig. 4). Another potential fate of glucose in the MGSKO mice is incorporation into liver glycogen; the *GYS2 gene*, encoding the liver isoform of glycogen synthase, is intact and liver glycogen synthase total activity was not affected in the null mice (23.5 ± 1.6 wild type, 23.0 ± 2.5, MGSKO nmol · $min^{-1}$ · $mg^{-1}$ protein, $n = 5$). In fed MGSKO mice, there was a trend for lower liver glycogen (377 ± 33 wild type, 297 ± 29 MGSKO μmol glucose/g tissue, $n = 8$, $P = 0.1$), whereas in fasted mice there was no significant difference (Fig. 5). Two hours after glucose injection, mice were killed for measurement of liver glycogen levels. In both the wild-type and MGSKO animals, ~12% of the injected glucose accumulated as liver glycogen (Fig. *5*), suggesting that increased synthesis of liver glycogen is not responsible for the enhanced glucose disposal by MGSKO mice. During the same GTT, there was no significant increase in muscle glycogen levels in the wild-type mice (7.4 ± 1.2 fasted, 5.9 ± 0.5 μmol glucose/g tissue after GTT), arguing against muscle glycogen being a major contributor to glucose disposal in mice.

**Insulin levels in MGSKO mice.** Fed male MGSKO mice have lower serum insulin levels as compared with wild-type controls (Table 1), which would be consistent with enhanced insulin sensitivity. However, insulin tolerance tests, in which blood glucose was monitored after intraperitoneal insulin injection, indicated that both male and female null mice responded similarly to their wild-type and heterozygous littermates (data not shown). Insulin levels were similar in wild-type and MGSKO mice fasted for 6 h (0.85 ± 0.11 wild type, 1.10 ± 0.15 ng/ml MGSKO, $n = 8$) or overnight (0.68 ± 0.09 wild type, 0.55 ± 0.06 ng/ml MGSKO, $n = 5$–7). After administration of glucose during a GTT, the initial increase in serum insulin was similar in wild-type and MGSKO mice but remained higher for an extended period of time in MGSKO mice (Fig. 5), correlating with the reduced circulating glucose levels (Figs. 4, 5). This result argues that either insulin secretion or clearance is modified in the MGSKO mice.

**Euglycemic-hyperinsulinemic clamp.** As a further exploration of glucose metabolism and insulin sensitivity in MGSKO mice, animals were subjected to euglycemic-

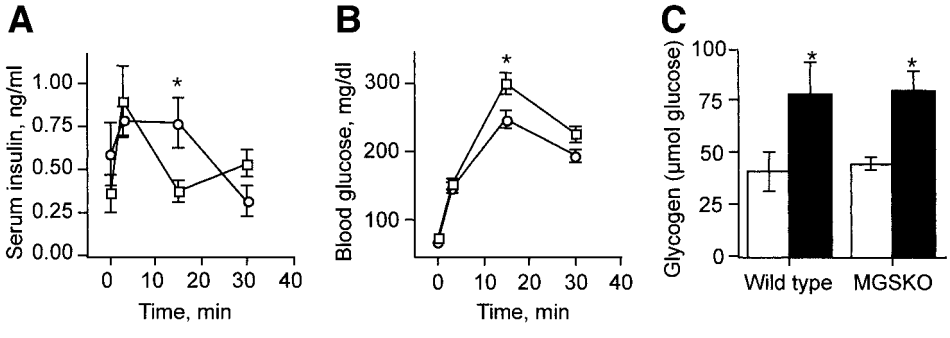


**FIG. 5. Serum insulin levels during and liver glycogen content after a GTT. Female wild-type (□) and MGSKO (○) mice (16–18 months of age) were subjected to a GTT, and serum insulin (*A*) and blood glucose (*B*) were monitored, $n = 10$. Liver glycogen (*C*) was measured in the fasted state (□) and 2 h after glucose injection (■). $n = 5$–7, *$P < 0.05$**

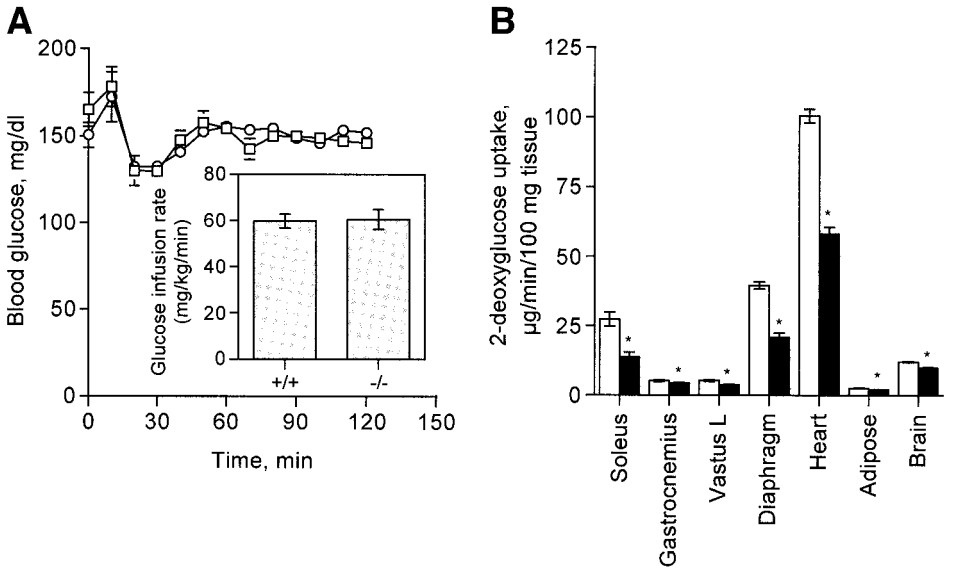


**FIG. 6. Glucose disposal rate and tissue-specific glucose uptake during a euglycemic-hyperinsulinemic clamp. *A*: Five-month-old male wild-type (□) and MGSKO (○) mice were subjected to the clamp with blood glucose maintained at ~150 mg/dl. *Inset*: Glucose infusion rate in wild-type and MGSKO mice during the clamp. *B*: After subjecting mice to the clamp for 2 h, tracer was administered for 25 min and uptake into several tissues was measured in wild-type (□) and MGSKO mice (■). $n$ = 8, *$P$ < 0.05 compared with wild type.**

hyperinsulinemic clamps. The glucose infusion rate was comparable in wild-type and null mice, indicating that whole-body glucose disposal and insulin sensitivity were not improved in MGSKO animals under the conditions of the clamp (Fig. 6). In fact, tissue-specific glucose uptake during the clamp was decreased in all tissues tested from MGSKO animals (Fig. 6) indicative of peripheral insulin resistance. As judged by Western analysis, total GLUT4 protein was the same in skeletal muscle from wild-type and MGSKO mice (data not shown). Liver glycogen in samples collected at the end of the clamp were significantly higher in MGSKO mice (59.1 ± 14.0 μmol glucose/g tissue) as compared with wild-type littermates (24.6 ± 7.5 μmol glucose/g tissue, $n$ = 8, $P$ = 0.048).

**High-fat feeding.** Placing mice on a high-fat diet is known to induce obesity and glucose intolerance (20). Both male and female wild-type and null MGSKO mice were subjected to a high-fat diet to determine whether the absence of glycogen affected the acquisition of glucose intolerance. Mice were started on the high-fat diet at 40 days of age and body weight, blood glucose, triglycerides, and blood lactate monitored biweekly. There were no differences in blood triglycerides or lactate levels between genotypes over the period that mice were on a high-fat diet (data not shown). As to be expected, body weight was significantly higher in both wild-type and MGSKO animals after 12 weeks on the high-fat diet as compared with age-matched animals on a standard diet (Fig. 7). As observed with standard feeding, MGSKO mice on a high-fat diet showed a trend to weigh less than wild-type mice on the same diet (Fig. 7). GTTs were performed 12 weeks into the study (Fig. 7). For both genotypes, fasting blood glucose was elevated as compared with control mice on a normal diet. Although the male MGSKO mice still tended to have improved glucose disposal, the difference was no longer statistically significant. In female MGSKO mice, however, their improved glucose disposal was preserved on the high-fat diet (Fig. 7). The lack of muscle glycogen can be partially protective against the insulin resistance induced by fat feeding.

## DISCUSSION

The role of skeletal muscle as a primary site of insulin-dependent glucose disposal in humans is well established (5,21). In mice, its role is less clear. A number of transgenic and knockout mouse models have targeted muscle glucose disposal. Muscle-specific disruption of the insulin receptor causes skeletal muscle insulin resistance without frank diabetes (22,23). Elimination of muscle GLUT4 leads to a more severe phenotype, with impaired muscle glucose uptake and the development of diabetes in a subset of the mice (24,25). Basal muscle glycogen levels in both models were normal but insulin-stimulated glycogen synthesis was reduced (22,25–27). In contrast, transgenic mice overexpressing GLUT4 (28,29) and GLUT1 (28,30,31) in muscle had increased glucose uptake, increased muscle glycogen content, and improved glucose tolerance. This improvement in glucose tolerance occurred despite skeletal muscle insulin resistance as determined by euglycemic-hyperinsulinemic clamp (28) and in ex-vivo muscle studies (31,32). These mouse models generally reveal a positive

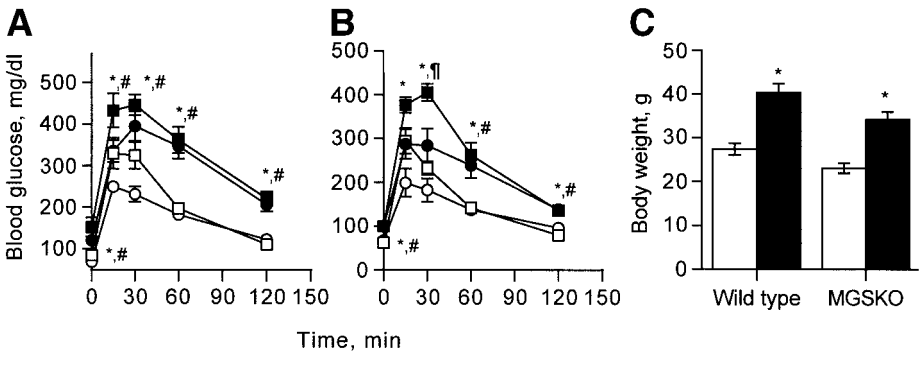


**FIG. 7. Glucose tolerance in mice on a high-fat diet. Five-month-old male (*A*) or female (*B*) wild-type (■) and MGSKO (●) mice were maintained on a high-fat diet for 12 weeks and subjected to a GTT. Wild-type (□) and MGSKO (○) mice on standard diet are shown for comparison. $n$ = 3–6, *$P$ < 0.05 comparing wild-type mice on high-fat diet versus standard diet, #$P$ < 0.05 comparing MGKSO mice fed a high-fat diet versus a standard diet. Body weight (*C*) of mice after 12 weeks of feeding either a normal diet (□) or a high-fat diet (■). $n$ = 6–8, *$P$ < 0.05 compared with same genotype on a normal diet.**

correlation between muscle glucose uptake and overall glucose tolerance but do not specifically address the role of muscle glycogen synthesis. Glycogen levels in mouse muscle have also been manipulated via type 1 protein phosphatase subunits that target the enzyme to glycogen. Two reports (8,33) describe disruption of the gene encoding $R_{GL}$ or $G_M$, which is expressed only in striated muscle, with conflicting results. In both cases, loss of $R_{GL}$ results in reduced muscle glycogen synthase activity and glycogen. Suzuki et al. (8) found no change in body weight, in vitro insulin-stimulated glucose uptake, or glucose tolerance as judged by GTT in three independently generated lines. Delibegovic et al. (2003) reported that the null mice had increased fat mass upon aging and developed glucose intolerance in a GTT (33). The latter study depended on a single embryonic stem cell line. Disruption of the gene encoding the ubiquitously expressed protein targeting to glycogen type 1 phosphatase glycogen-targeting subunit (34) was lethal, but heterozygotes for the null mutation had less active glycogen synthase and reduced glycogen stores in several tissues (35). These animals acquire insulin resistance and glucose intolerance with aging. In all of the preceding models, however, the mutation is upstream of glycogen biosynthesis, and other factors besides muscle glycogen could contribute to the phenotype. The MGSKO mice provide, in principle, a much stricter test of the importance of muscle glycogen for blood glucose homeostasis.

The phenotype of the MGSKO mice as related to glucose homeostasis was complex. The animals disposed of glucose better than wild-type animals in a GTT but, in a euglycemic-hyperinsulinemic clamp, insulin-sensitivity of MGSKO mice, judged by glucose infusion rate, was no different from wild type. Insulin-induced glucose uptake into peripheral muscle, brain, and adipose tissue was decreased whereas liver glycogen accumulation was enhanced in the MGSKO mice. A possible explanation for the decrease in glucose uptake is that glucose-6-P, unable to be converted to glycogen, builds up and inhibits hexokinase. The apparent conflict between the results of the GTT and the clamp can be explained in part by experimental conditions. During the clamp, ~1,250 μmol of glucose are delivered over a 2.25–h period with insulin maintained at an elevated level (2.5 ng/ml) and, despite insulin resistance of peripheral tissues, enhanced glycogen synthesis in the liver of the MGSKO mice contributes to maintaining normal insulin sensitivity. During a GTT, ~280 μmol of glucose are disposed in ~120 min with serum insulin free to increase but still remaining below ~1 ng/ml. No change in glycogen synthesis by the liver is observed. Rather, increased glucose disposal by the MGSKO mice correlates with more sustained elevation in serum insulin during the GTT, a response not achievable with the clamp protocol where the insulin concentration is fixed.

Since the absence of muscle glycogen did not impair glucose disposal, one might question the importance of muscle glycogen accumulation for blood glucose homeostasis in the mouse. This conclusion is reinforced by the fact that, over the course of a GTT, there is no net accumulation of glycogen in the skeletal muscle of wild-type mice. After the 2-h period, however, the equivalent of 12% of the injected glucose was measured as liver glycogen in both genotypes. The fate of the remainder of administered glucose has not been quantitated in the current study. However, our calculation does not take into account storage of glucose equivalents in other tissues as glycogen, fat, or, perhaps more importantly, glucose utilization. If one considers an ~15-kcal daily caloric intake for a 25-g mouse (8), on average the mouse consumes 0.625 kcal per hour. Therefore, it is not unreasonable to expect that a significant fraction of the injected glucose, caloric equivalent of 0.204 kcal, based on 4 kcal/g, is simply consumed during the GTT by the normal activity of the mice. In several clamp studies, it has been proposed that glucose consumption by glycolysis in muscle is much greater than disposal as glycogen (for example 36,37). If all of the injected glucose (~280 μmol for a 25-g mouse) was converted to muscle glycogen, we would anticipate an increase in muscle glycogen of ~33 μmol glucose/g tissue, assuming that 30% of the body weight is muscle. The basal amount of muscle glycogen, with the same assumptions, is 50–80 μmol glucose equivalents compared with 400–500 μmol glucose equivalents of liver glycogen. Thus, in fed mice, there is 5–10 times as much liver glycogen as muscle glycogen, and the former may play the dominant role in glucose disposal. The importance of the liver generally to blood glucose homeostasis is emphasized by the phenotype of the LIRKO mouse, in which the insulin receptor is specifically disrupted in the liver (38). These animals are hyperglycemic, glucose intolerant, and insulin resistant.

The foregoing reasoning does not mean that muscle glycogen is not important for glucose disposal and insulin resistance in humans. As a fraction of body weight, human muscle glycogen is ~10 times greater than mouse muscle glycogen (our results, 39). Both the muscle glycogen concentration, 80–100 μmol glucose/g (40), and muscle mass as a fraction of body weight, ~40% (41,42), are greater in humans, and muscle glycogen has a more central role in glucose homeostasis. Also, in contrast to mice, humans have three- to eightfold more muscle glycogen than liver glycogen (43). Therefore, defects in mouse models that cause relatively modest impairments in muscle metabolism or glucose uptake without causing diabetes may well translate into a more severe phenotype in humans. In fact, the demonstration that skeletal muscle is insulin resistant in the MGSKO mice makes an argument that impairment of the ability to synthesize muscle glycogen in humans could well make a significant contribution to glucose intolerance.

Although the absence of a muscle glycogen store in MGSKO mice can have a direct impact on glucose metabolism, experience with the numerous mouse lines carrying defects in insulin signaling and intermediary metabolism cautions that genetic interventions can cause complex and indirect effects as well. Adaptations can lead to metabolic changes in the affected tissue as well as other organs, whether by modifying fuel-usage patterns or altering communication among organs. In the case of MGSKO mice, 90% of the null pups die shortly after birth, raising the question of a secondary factor that permits survival of the subset of mice analyzed in the current study. As previously described (9), the death of these newborns appears to be due, at least in part, to defects in heart development. However, survivors have hearts that are relatively normal and slightly larger. One important compensatory mechanism in MGSKO mice is the modification in fatty acid oxidation; however, the fact remains that improved glucose tolerance and sustained elevation of serum insulin concentration during the GTT occur in animals that are devoid of skeletal muscle glycogen. In the MGSKO animals, the absence of glycogen correlates with development of muscle that appears less fast glycolytic and more

oxidative. Increased phospho-ACC and phospho-AMPK suggests greater oxidative capacity in muscle and may account, at least in part, for the improved glucose tolerance observed in MGSKO animals and the reduced fat mass in these mice. The β-subunit of AMPK contains a carbohydrate-binding domain (44) that binds glycogen, although the functional significance of this interaction is not fully understood (45,46). There are reports, however, that elevated muscle glycogen correlates with suppression of AMPK activity (45,47). Together with our finding that lack of glycogen activates AMPK, one might propose that glycogen inhibits AMPK in muscle as part of the nutrient-sensing process. Phosphorylation of ACC is inactivating and so ACC gene disruption should partly mimic the increased ACC phosphorylation observed in the muscle of MGSKO mice. Interestingly, ACC2-null (48) and MGSKO mice share protection against high-fat feeding–induced glucose intolerance.

In some mouse models with impaired muscle glucose metabolism, secondary effects on liver, adipose tissue, and other organs can occur. For example, the MIRKO mice develop greater adiposity (49). Muscle-specific disruption of GLUT4 results in a significant increase in liver glucose uptake and glycogen accumulation, suggesting that glucose unable to enter the muscle is shunted to the liver (24). In mice lacking GLUT4 in both adipose tissue and muscle, the liver assumes a much greater role in glucose homeostasis, in part by increased fat metabolism (50). Liver glycogen levels were not reported in this study. In any event, MGSKO mice may have a milder version of these phenotypes, with an enhanced role for liver apparent only under the forcing conditions of the euglycemic-hyperinsulinemic clamp when increased glycogen accumulation was observed. However, this reasoning cannot easily explain the improved glucose disposal by MGSKO mice during a GTT. The strongest clue from the present work is the sustained elevation of serum insulin coinciding with lowered blood glucose. There are several possibilities that could explain these data. First, muscle could be influencing insulin levels by communicating with β-cells to increase insulin secretion or with the liver to suppress insulin breakdown. Indeed, the ability of muscle to communicate with pancreatic β-cells was already proposed by Mauvais-Jarvis et al. (51). In MGSKO mice, this interorgan communication would have to be affected by the presence of glycogen in muscle. There is a precedent for muscle glycogen content affecting the formation and release of regulatory molecules in that exercise-induced interleukin-6 release by the muscle is enhanced as glycogen levels are decreased (52). From study of MGSKO mice, we have not however noted any significant effects of the loss of muscle glycogen on either basal or exercise-induced serum interleukin-6 levels (B.A.P., M.W.S., C.R. Cope, J.M.S., P.J.R., unpublished observations). An alternative explanation is that disruption of *GYS1* in some tissue other than muscle causes the improved glucose tolerance. *GYS1* is expressed in brain and, if brain glycogen is implicated, it is not through control of appetite since the MGSKO mice have normal food consumption. Nor is loss of expression of glycogen synthase in adipose tissue a likely explanation for improved glucose tolerance since insulin-stimulated glucose uptake is reduced in fat of MGSKO animals. With modified insulin secretion as one potential explanation for the performance of MGSKO mice in GTTs, an interesting hypothesis is that *GYS1* is expressed in β-cells and that the absence of glycogen promotes insulin release. We can measure glycogen synthase activity and mRNA in the pancreas of wild-type mice, but neither is detectable in MGSKO animals (B.A.P., G.E.P., P.J.R., unpublished observations). The next step is to establish whether the enzyme is present in the β-cells. Glucokinase is considered to be important for glucose sensing and one speculation is that, without glycogen as a glucose sink, glucose-6-P or other metabolites take longer to be metabolized and so prolong signals for insulin secretion. Also, like skeletal muscle, perhaps β-cells have increased oxidative capacity in MGSKO mice leading to an elevated ATP-to-ADP ratio in response to a given glucose load. Further studies will address these possibilities.

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