Exercise training does not correct abnormal cardiac glycogen accumulation in the \textit{db/db} mouse model of type 2 diabetes

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Shearer J, Ross KD, Hughey CC, Johnsen VL, Hittel DS, Severson DL. Exercise training does not correct abnormal cardiac glycogen accumulation in the \textit{db/db} mouse model of type 2 diabetes. Am J Physiol Endocrinol Metab 301: E31–E39, 2011. First published March 8, 2011; doi:10.1152/ajpendo.00525.2010.—Substrate imbalance is a well-recognized feature of diabetic cardiomyopathy. Insulin resistance effectively limits carbohydrate oxidation, resulting in abnormal cardiac glycogen accumulation. Aims of the present study were to 1) characterize the role of glycogen-associated proteins involved in excessive glycogen accumulation in type 2 diabetic hearts and 2) determine if exercise training can attenuate abnormal cardiac glycogen accumulation. Control (\textit{db}+) and genetically diabetic (\textit{db/db}) C57BL/\textit{KsJ-lepr\textasciidoth/dlep} mice were subjected to sedentary or treadmill exercise regimens. Exercise training consisted of high-intensity/short-duration (10 days) and low-intensity/long-duration (6 wk) protocols. Glycogen levels were elevated by 35–50% in \textit{db/db} hearts. Exercise training further increased (2- to 3-fold) glycogen levels in \textit{db/db} hearts. Analysis of soluble and insoluble glycogen pools revealed no differential accumulation of one glycogen subspecies. Phosphorylation (Ser⁶⁴⁰) of glycogen synthase, an indicator of enzymatic fractional activity, was greater in \textit{db/db} mice subjected to sedentary and exercise regimens. Elevated glycogen levels were accompanied by decreased phosphorylation (Thr¹⁷²) of 5′-AMP-activated kinase and phosphorylation (Ser⁷⁹) of its downstream substrate acetyl-CoA carboxylase. Glycogen concentration was not associated with increases in other glycogen-associated proteins, including malin and laforin. Novel observations show that exercise training does not correct diabetes-induced elevations in cardiac glycogen but, rather, precipitates further accumulation.

proglycogen; metabolism

One striking change related to substrate imbalance in the diabetic heart is a two- to threefold increase in glycogen concentration (29). Although once considered benign and simply a by-product of perturbed metabolism, recent findings show glycogen to be a central factor in the regulation of cellular homeostasis influencing key signaling pathways, substrate uptake and oxidation, contractility, and normal patterns of gene expression (42). Of particular interest, glycogen is now well recognized as a potent regulator of 5′-AMP-activated protein kinase (AMPK). This serine/threonine stress kinase is activated by increasing ratios of AMP to ATP and glycogen depletion (27). Activation of the enzyme via phosphorylation results in stimulation of energy production pathways during simultaneous inhibition of energy-consuming processes. AMPK is essential to the heart and its ability to survive energy deficits imposed by cardiac injury. Mice with a mutant form of the protein fail to increase glucose and fatty acid utilization and have lower ATP concentrations and sustain greater injury in response to ischemia compared with their wild-type counterparts (30). Similarly, mutations in AMPK cause glycogen storage cardiomyopathy (33).

Although exercise is commonly prescribed for insulin resistance, the impact of exercise training on cardiac glycogen and AMPK is poorly understood. Exercise is known to modulate glycogen stores, as well as AMPK activity, in this tissue (25). Given this background, objectives of the present study are to 1) characterize the molecular targets responsible for elevated glycogen accumulation in the diabetic heart and 2) determine if exercise training can attenuate glycogen accumulation in cardiac muscle. Studies were executed using the type 2 diabetic (\textit{db/db}) mouse model. This monogenic model displays early obesity with peripheral insulin resistance followed by hyperinsulinemia and hyperglycemia.

METHODS

Mouse Maintenance

Procedures were approved by the University of Calgary Animal Care and Use Committee and conform to the Canadian Association for Laboratory Animal Science guidelines for animal experimentation. Animals were maintained in a humidity-controlled room with a 12:12-h light-dark cycle and were fed a standard laboratory chow (Purina, Richmond, IN). Control (heterozygote, \textit{db}+) and C57BL/\textit{KsJ-lepr\textasciidoth/dlep} (\textit{db/db}) mice were purchased from Jackson Laboratories (Bar Harbor, ME). Diabetic \textit{db/db} mice express an early phenotype of insulin resistance, detectable at 10 days of age by hyperinsulinemia, with beta cell hyperplasia and hypertrophy. By 12 wk of age, \textit{db/db} mice exhibit a severe type 2 diabetic phenotype, with marked obesity and profound hyperglycemia.

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Exercise Training

High-intensity/short-duration (10 days) protocol. Mice of each genotype (db+ and db/db) were randomly sorted into sedentary or exercise treatments. Mice were 12 wk of age at the start of the protocol. Exercise was performed on a rotating treadmill-wheel system (Lafayette Instruments, Lafayette, IN). To account for stress induced by animal handling, sedentary animals were placed in a stationary treadmill for acclimation and exercise treatments. Acclimation to exercise consisted of 15 min of treadmill exercise at 5.64 m/min for 3 days in exercise-treated animals followed by a 3-day rest period. Exercise was performed at a constant intensity for a defined duration, 5.64 m/min, 2 h/day for 10 consecutive days. Total distance over 10 days was 6,788 m, which matched the low-intensity/long-duration protocol [see Low-intensity/long-duration (6 wk) protocol].

Low-intensity/long-duration (6 wk) protocol. To examine the long-term effects of exercise training on cardiac glycogen accumulation, a second group of mice was examined. Mice started the protocol at 6–7 wk of age and were 12–13 wk old when they were killed.

For mice in this group, the above-described procedures [see High-intensity/short-duration (10 days) protocol], differing only in exercise intensity and duration (5.33 m/min, 60 min/day, 5 days/wk for a total of 6 wk), were followed. Total distance over 6 wk was 6,716 m, which was matched in the high-intensity/short-duration protocol.

Animal Experimentation

All mice were the same age at the time of tissue collection. At the end of the exercise period, animals were rested for 24–36 h before they were killed to ensure that hearts were glycogen-replete. In previous work, 18 h after the last bout of exercise was shown to be sufficient for the acute effect of exercise on insulin responsiveness to wear off (9). This time included 6 h of fasting to minimize the effects of postprandial food absorption. Animals were then weighed and anesthetized (pentobarbital sodium). Whole blood (1 ml) was obtained from animals prior to storage at 80°C. Blood glucose was assessed routinely (One Touch, Lifescan, Bunaby, BC, Canada). Nonesterified fatty acids (NEFAs) were measured spectrophotometrically (NEFA C kit, Wako, Richmond, VA). Immunoreactive insulin was assayed with a double-antibody method (24). Cardiac tissue was excised and briefly rinsed in saline. As glycogen is prone to rapid degradation, ventricles were isolated and rapidly freeze-clamped in liquid nitrogen and stored at −80°C for further analysis.

Glycogen Determination

Glycogen was assessed as acid-soluble and -insoluble fractions, as previously described (3). Briefly, acid-insoluble and -soluble fractions were separated by addition of 1.5 M perchloric acid to a 10-mg sample of freeze-dried tissue. Once glycogen was separated into acid-soluble and -insoluble fractions, glucosyl units were determined by enzymatic measurement [Glucose (GO) kit, Sigma Chemical, St. Louis, MO]. Total glycogen was calculated as the sum of measured acid-soluble and -insoluble glycogen.

Glucose 6-Phosphate Analysis

Cardiac tissue (~10 mg) was homogenized in ice-cold 1× PBS and then deproteinized using a perchloric acid-KOH deproteinizing sample preparation kit (BioVision, Mountain View, CA). Glucose 6-phosphate (G6P) levels were measured using a standard kit (BioVision). According to the manufacturer’s instructions, sample homogenates were added to a 96-well plate with enzyme and substrate components and incubated for 30 min at room temperature. The reaction was quantified using a microplate reader (SpectraMax, Molecular Devices, Sunnyvale, CA) at 450 nm.

Quantitative Real-Time PCR

Total RNA was extracted from samples with TRIzol reagent (Invitrogen, Carlsbad, CA). This was followed by reverse transcription employing a standard kit (First-Strand cDNA Synthesis Kit for RT-PCR, Invitrogen). A total of 1 µg of RNA was used with oligo(dt)18 as a primer. The resultant cDNA was amplified using custom primers generated by Invitrogen and analyzed using quantitative RT-PCR (qRT-PCR). All primer sequences are shown in Table 1. qRT-PCR were heated for 2 min at 95°C, then 40 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, in a real-time thermal cycler (model CFX96, C1000, Bio-Rad, Hercules, CA). GADPH and β-actin were verified as suitable housekeeping genes for cardiac muscle, and primers were included as an internal control in all reactions. The 2−ΔΔCT method was utilized for data analysis, where threshold cycle (Ct) indicates the fractional cycle number at which the amount of amplified target reaches a fixed threshold (8). ΔΔCt is the difference in threshold cycles for the gene of interest and GADPH, and ΔΔCt is the difference in ΔCt between treatment groups. Relative expression levels are presented as fold changes to the sedentary db+ group, for which levels were set to 1.

Table 1. Primer sequences for quantitative RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>PCR Size, bp</th>
</tr>
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<tbody>
<tr>
<td>GLN1</td>
<td>GATGCGACCTCCTACTCTAATGATG  TCCACGCGTTCCCTCCTTTC</td>
<td>122</td>
</tr>
<tr>
<td>Malin</td>
<td>TCCTGCTGACTGAGCTGGAG  AGTCACAGGAGGACTTT</td>
<td>297</td>
</tr>
<tr>
<td>Laforin</td>
<td>TGACAGCGACCCAGACATTTA  GATTTTCTGGTTCTCTTGC</td>
<td>288</td>
</tr>
<tr>
<td>GSY1</td>
<td>GAGACCGACCCAGTGTTTCCA  CGCAAGCTGCAACAACGTC</td>
<td>285</td>
</tr>
<tr>
<td>GSK3</td>
<td>TCACTACTCTCTGGAATGTGC  CACCAATGACGACACATG</td>
<td>80</td>
</tr>
<tr>
<td>PDK4</td>
<td>TCCACACCTGACGACAGTGC  AGAGACGCTTGGTTCAGTT</td>
<td>150</td>
</tr>
<tr>
<td>H-FABP</td>
<td>GACGCTGTCACGACATGA  TGGACGTTGAGGAGTACG</td>
<td>150</td>
</tr>
<tr>
<td>MCAD</td>
<td>AGCTGCTGACAGGGAGGATGC  AGGTGCTGCAATGACGG</td>
<td>272</td>
</tr>
<tr>
<td>ACS</td>
<td>ATTCGCCATACCCAGCATGA  CGTGACGCTGCTGAGC</td>
<td>235</td>
</tr>
<tr>
<td>UCP3</td>
<td>ACCACTCTCGTCTCTTC  GTATAGGCGCTCAAACAGA</td>
<td>189</td>
</tr>
<tr>
<td>β-Actin</td>
<td>CCTGCTGCAAGGAGACACT  GGGGTTGCTAGGAGTGG</td>
<td>165</td>
</tr>
</tbody>
</table>

GLN1, glycogenin 1; GSY1, glycogen synthase 1; GSK3, glycogen synthase kinase 3; PDK4, pyruvate dehydrogenase kinase 4; H-FABP, heart fatty acid-binding protein; MCAD, medium- or long-chain acyl-CoA dehydrogenase; ACS, acyl-CoA carboxylase; UCP3, uncoupling protein 3.
Protein Determination

Cardiac lysates in Laemmli buffer were separated by SDS-PAGE. Samples were resolved on 4–12% Bis-Tris SDS-polyacrylamide gels (Invitrogen) and then electrophoretically transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA). Membranes were blocked in 2% nonfat milk diluted in Tris-buffered saline (TBS) containing 0.05% Tween 20. Membranes were probed with primary antibodies overnight at 4°C and then incubated with secondary antibodies for 1 h at room temperature. Primary antibodies are as follows: laforin (catalog no. sc-70293, Santa Cruz Biotechnologies, Santa Cruz, CA), malin (catalog no. sc-67360 Santa Cruz Biotechnologies), AMPK (catalog no. 2532, Cell Signaling Technology, Boston, MA), phosphorylated (Thr172) AMPK (catalog no. 2531, Cell Signaling Technology), acetyl-CoA carboxylase (ACC; catalog no. 3662, Cell Signaling Technology), phosphorylated (Ser79) ACC (catalog no. 3661, Cell Signaling Technology), glycogen synthase (catalog no. 3891, Cell Signaling Technology), hexokinase II (catalog no. AB1629, Millipore, Billerica, MA), glucose transporter (GLUT) 1 (catalog no. ab652 Abcam, Cambridge, MA), Akt (catalog no. 4691, Cell Signaling Technology), phosphorylated (Ser473) Akt (catalog no. 4060, Cell Signaling Technology), glycogen synthase kinase (GSK) 3β (catalog no. 9315, Cell Signaling Technology), phosphorylated (Ser21) GSK3 (catalog no. 3931, Cell Signaling Technology), β-actin (catalog no. A2172, Sigma Chemical), and GADPH (catalog no. ab9485-100, Abcam). Membranes were washed and incubated in 2% nonfat milk diluted in TBS containing 0.05% Tween 20. Densitometry was performed using GeneTools (Syngene).

Statistical Analysis

All data are presented as means ± SE. A two-way ANOVA was performed to detect statistical differences (P < 0.05). Differences within the ANOVA were determined using Tukey’s post hoc test. Statistical analyses were performed using SigmaStat software (SPSS, Chicago, IL).

RESULTS

Body Mass

Mouse characteristics are reported in Table 2. Body mass was greater for db/db than db+ mice. Body mass was not altered by the high-intensity/short-duration or low-intensity/long-duration regimen (P > 0.05).

Cardiac Glycogen

Cardiac glycogen levels were determined in freeze-clamped ventricular tissue. Results are shown in Fig. 1A. In sedentary animals, cardiac glycogen levels were increased 35–50% in db/db compared with db+ animals (P < 0.05). Exercise training did not change cardiac glycogen levels in db+ animals (P > 0.05). However, in db/db hearts, exercise training further exacerbated (2- to 3-fold increase) elevations in cardiac glycogen (P < 0.05). Evaluation of the ratio of acid-insoluble to -soluble glycogen (also referred to as pro- and macroglycogen) was not different in the high-intensity/short-duration protocol (P > 0.05; Table 3). However, the percentage of glycogen found in the acid-insoluble fraction decreased in db/db mice with the low-intensity/long-duration exercise protocol (P < 0.05; Table 3). As the high-intensity/short-duration and low-intensity/long-duration exercise regimens had similar effects on cardiac glycogen (P > 0.05), only the high-intensity/short-duration group was chosen for further analysis. As such, all further measurements were performed on the high-intensity/short-duration group only.

Metabolic Characteristics

As expected, arterial blood glucose and plasma insulin levels were elevated in db/db compared with db+ mice (P < 0.05; Fig. 1B and C). Glucose levels were artificially high because of animal stress and the presence of anesthesia. Exercise training did not alter resting insulin or glucose levels (P > 0.05, 24–36 h postexercise). Plasma NEFAs were elevated in sedentary db/db animals but normalized with exercise training (P < 0.05; Fig. 1D). Lastly, examination of tissue concentrations of G6P, an allosteric activator of glycogen synthase, showed no difference between db+ and db/db hearts in the sedentary condition (P > 0.05). However, with exercise training, a difference between genotypes became apparent with greater concentrations of G6P in db/db than db+ hearts (P < 0.05; Fig. 1E).

qRT-PCR

To evaluate the molecular mechanisms involved in glycogen regulation in the heart, qRT-PCR was performed on key metabolic genes involved in insulin signaling and carbohydrate and fatty acid metabolism (Fig. 2). The combination of insulin resistance (db/db) and exercise training had no appreciable additive effect on any of the genes involved in glycogen metabolism, with the exception of glycogen synthase (P < 0.05).

When the effects of genotype (db+ vs. db/db) were examined within sedentary and exercise treatments, genes involved in fatty acid metabolism, including medium- and long-chain acyl-CoA dehydrogenase (MCAD) and heart fatty acid-binding protein (H-FABP), were elevated along with GLUT1 and other genes involved in carbohydrate metabolism.
malin \((P < 0.05)\). Pyruvate dehydrogenase kinase 4 and uncoupling protein 3 levels were greater in sedentary \(db/db\) than \(db^+\) animals \((P < 0.05)\). With exercise treatment, these differences between genotypes were no longer apparent \((P > 0.05)\). Lastly, the serine/threonine protein kinase Akt was increased with exercise training in \(db^+\) and \(db/db\) animals.

**Immunoblotting**

Evaluation of a number of proteins involved in the regulation of glucose uptake and glycogen storage was evaluated. Levels of total proteins (unphosphorylated), \(\beta\)-actin, and GADPH did not change with genotype or treatment and were used as loading controls \((P > 0.05)\). All graphical data were normalized to the respective protein or GADPH. Protein levels of hexokinase II, which is primarily responsible for glucose trapping, remained unchanged \((P > 0.05)\). Measurement of glycogen synthase phosphorylation \((\text{Ser}^{640})\), a surrogate marker of fractional activity \((28)\), was greater in \(db/db\) hearts from sedentary and exercised animals \((P < 0.05; \text{Fig. 3B})\). Total glycogen synthase protein did not change.

**Table 3.** Percent total glycogen in acid-insoluble, proglycogen form in sedentary and exercised \(db^+\) and \(db/db\) mice

<table>
<thead>
<tr>
<th></th>
<th>High-Intensity/Short-Duration ((n = 10))</th>
<th>Low-Intensity/Long-Duration ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(db^+)</td>
<td>(db/db)</td>
</tr>
<tr>
<td>Sedentary</td>
<td>59 ± 8</td>
<td>39 ± 6</td>
</tr>
<tr>
<td>Exercise</td>
<td>53 ± 4</td>
<td>37 ± 2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Animals were subjected to high-intensity/short-duration regimen for 10 days and to low-intensity/long-duration regimen for 6 wk. Remaining glycogen was found in larger, acid-soluble macroglycogen form. *\(P < 0.05\) vs. \(db^+\) within a treatment.

**Fig. 1.** Insulin resistance and exercise training induce abnormal glycogen accumulation in hearts from \(db/db\) mice. A: total cardiac glycogen levels in \(db^+\) and \(db/db\) mice in sedentary and exercised \((10 \text{ days, } n = 10/\text{group})\) and low-intensity/long-duration \((6 \text{ wk, } n = 5)\) groups. Insulin resistance \((db/db\) resulted in elevated cardiac glycogen, while insulin resistance + exercise further augmented glycogen storage. B–D: plasma glucose, insulin, and nonesterified fatty acid (NEFA) levels. E: concentration of glucose 6-phosphate (G6P) in the heart. Values are means ± SE. *\(P < 0.05\) vs. \(db/db\) within sedentary or exercise treatments. **\(P < 0.05\) vs. all other treatments. In B–E, values represent 10 animals per treatment (high-intensity/short-duration exercise group only).
phosphorylation causes inactivation of this enzyme. Glycogen-associated proteins, including malin and laforin, were also quantified, and no differences were observed between genotypes or treatment (Fig. 3, C and D). To investigate the role of GSK3β, phosphorylation of Ser9 was detected. Results show no difference in phosphorylation at this site with exercise training (Fig. 3E). Results demonstrate that exercise in db/db animals causes an elevation in cardiac G6P levels, we determined levels of GLUT1 (Fig. 3F). Neither the db/db genotype nor exercise had an appreciable effect on this protein. Phosphorylation of AMPK and ACC was blunted in hearts from db/db animals (P < 0.05, Fig. 4).

DISCUSSION

Goals of the present study were to examine the molecular determinants of diabetes-induced glycogen accumulation and to determine whether exercise training could reverse abnormal glycogen accumulation in a model of diabetic cardiomyopathy. Similar to humans, the db/db mouse model of type 2 diabetes displays obesity, hyperinsulinemia, insulin resistance, and hyperglycemia (34). Examination of contractile function in isolated perfused db/db hearts (6, 7) also shows impaired ventricular function and hypertrophy, making hearts from db/db mice an ideal experimental model for this purpose.

Results demonstrate greater accumulation of cardiac glycogen in db/db mice than in their db+ counterparts. Surprisingly, exercise training at two different intensities and durations did not normalize cardiac glycogen but, rather, resulted in a further increase in glycogen stores, proving that our initial hypothesis was incorrect. To determine the mechanisms involved in glycogen accumulation in this model, acid-insoluble and -soluble glycogen fractions were determined. Commonly referred to as pro- and marccoglycogen, these subspecies can be separated by their differential protein-to-carbohydrate ratio (17) and, possibly, their association with cytoskeletal elements (18). In skeletal muscle, these two forms of glycogen have different metabolic sensitivities, with acid-insoluble glycogen being more sensitive to degradation (36). It has been suggested that acid-insoluble glycogen may be preferentially localized to the sarcoplasmic reticulum and involved in the provision of ATP for Ca2+ handling (26). The ratio of acid-soluble to -insoluble glycogen provides insight into whether augmented glycogen stores result from the addition of new glycogen granules or the growth of existing molecules. Results of the present study show a lower percentage of acid-insoluble glycogen in db/db animals in the low-intensity/long-duration treatment group. Similar, albeit nonsignificant, changes are seen in the high-intensity group, but with much larger variability (P > 0.05). These results suggest that glycogen granules in the heart are not more numerous but, rather, larger in size.

To examine the mechanisms involved in abnormal cardiac glycogen accumulation in diabetes, a number of key regulatory steps in glycogen synthesis were examined. Starting with glucose uptake, we show no change in hexokinase II protein. While glucose transport (GLUT1 and GLUT4) is the primary barrier to muscle glucose uptake under basal conditions, glucose phosphorylation by hexokinase II is limiting to muscle glucose uptake during stimulated conditions such as hyperinsulinemia or exercise (14). In contrast to mouse models of diet-induced insulin resistance (15), we show no effect of the db/db genotype on relative levels of cardiac hexokinase II compared with the db+ genotype in sedentary and exercise treatments. Next, levels of G6P were determined, as this metabolite is a potent allosteric activator of glycogen synthase. Levels of G6P were greater in exercise-trained db/db than db+ animals. This indicates that exercise lowered G6P levels in db+, but not db/db, animals. Examination of GLUT1 confirmed that changes in G6P were not due to increases in this protein.

Next, glycogen synthase, a commonly regarded rate-limiting enzyme for glycogen synthesis, was examined. Glycogen synthase undergoes allosteric activation by G6P and covalent
phosphorylation at nine sites, allowing for numerous phosphorylation configurations.

In the present study levels of total glycogen synthase did not change with genotype or treatment. The activity of glycogen synthase was assessed by examining phosphorylation at a single site (site 3a) corresponding to Ser640. This site was chosen, as it most closely reflects fractional activity of the enzyme and is regulated by glycogen content (21). Results of the present study show that insulin resistance (db/db) causes phosphorylation of glycogen synthase at Ser640. We show that a number of signals, including an elevation in glycogen itself and glycogen synthase phosphorylation, are acting to suppress glycogen synthase. Despite these signals, allosteric activation of glycogen synthase by G6P seems to predominate, ridding the cardiomyocyte of excess glucose. Indeed, there is strong evidence that phosphorylation of a single site does not prevent glycogen synthase activation (20, 37). From the findings cited above, it is apparent that the need to protect the cell from glucose accumulation overrides glycogen synthase inhibition. Another possibility is that glycogen phosphorylase activity was impeded by elevated insulin levels in the db/db model. In this scenario, lower glycogen phosphorylation would explain the differences in cardiac glycogen between genotypes.

Probing further into the mechanisms of glycogen regulation in diabetes, we also assessed two novel proteins, malin and laforin. To our knowledge, this is the first examination of these proteins in a diabetic state in the heart. Laforin is a dual-specificity phosphatase that acts to regulate glycogen synthesis and degradation (40). The protein facilitates interactions between protein phosphatase 1, glycogen synthase, and glycogen granules (13). Malin is a newly identified E3 ubiquitin ligase that interacts with laforin. Malin regulates glycogen metabo-

Fig. 3. Protein determination in db+ and db/db hearts at rest and following high-intensity/short-duration exercise. A: hexokinase II (HK II). B: phosphorylated (Ser640) glycogen synthase (GS). C: laforin. D: malin. E: phosphorylated (Ser640) GS kinase 3β (p-GSK3β). F: GLUT1.

Representative immunoblots are shown for each protein. All values are normalized to GAPDH; β-actin was used as a secondary control. All measures were obtained from the left ventricle. Values are means ± SE of 7–8 animals per treatment. *P < 0.05 vs. db+ within sedentary or exercise treatments.
lism by targeting and ubiquitinating numerous glycogen-associated proteins, including laforin (16). In this capacity, it acts as a gatekeeper, controlling glycogen metabolism by limiting glycogen accumulation (44). Results of the present study demonstrate no change in laforin or malin (mRNA and protein) with diabetes or exercise training. Cumulatively, our results suggest that abnormal glycogen accumulation in cardiomyopathy is not due to deviations in glycogen structure or synthesis.

While the mechanisms by which glycogen accumulates in the diabetic heart are of interest, so are its implications on survival following a cardiac event. Lowering glycogen levels not only reduces infarct size but also enhances functional recovery postischemia (5, 43). This phenomena appears to be due to a close interdependence of glycogen, glycogen-metabolizing proteins, and AMPK (31, 38). AMPK has a glycogen-binding domain and is modulated by the malin-laforin complex (23, 38). During ischemia, AMPK activation results in the stimulation of energy production to maintain ATP within a tight range, essential for contractile function. The protein also appears to aid recovery and limit cardiac remodeling following a cardiac event (10). In the present study, elevated glycogen levels in db/db hearts resulted in blunted AMPK phosphorylation, with and without exercise training. This inhibition was mimicked in the downstream AMPK target, ACC. Although not investigated in the present study, the combination of elevated glycogen levels and depressed AMPK activity would be anticipated to exaggerate cardiac injury following a cardiac event. Results show that exercise training, at the intensities employed in this study, was unable to correct these deficits.

Study limitations include an inability to fully explain the cause of increased glycogen accumulation in db/db mice. Our working hypothesis is that glycogen accumulates in db/db mice because of a lack of glucose oxidation, as documented in previous studies from our laboratory (2). Unfortunately, in vivo measures of glucose oxidation are not possible, and isolation of the heart would likely cause differences in glycogen between treatments to be lost. Similarly, the process of isolating cardiomyocytes causes significant glycogen depletion. Factors such as insulin resistance, time course of diabetic cardiomyopathy, and intensity and duration of exercise could also alter results. Exercise intensities were chosen so that all mice could complete the exercise protocol without injury and excessive stress (compared with methods involving forced swimming and electric shock). Although mice did not lose mass with exercise training, the protocol was effective at normalizing plasma NEFA levels, as well as uncoupling protein 3, Akt, and pyruvate dehydrogenase kinase 4, genes known to perpetuate metabolic dysfunction in insulin resistance. No difference in plasma glucose or insulin was observed, likely because of the highly insulin-resistant state of the db/db mouse. After exercise, animals were also rested for 36 h, a duration that would be expected to negate any effects of exercise itself on insulin sensitivity and AMPK signaling (25).

In summary, we show that the db/db model of type 2 diabetes has abnormal levels of cardiac glycogen that are not corrected with exercise training. This accumulation is undoubtedly detrimental to the heart, perpetuating insulin resistance and AMPK downregulation. Our findings show that glycogen structure, ratio, and assembly and key proteins involved in glycogen metabolism are predominantly maintained in diabetic cardiomyopathy. Rather, we hypothesize that excessive glycogen accumulation in diabetes may be due to defects in glucose oxidation, as previously shown by our laboratory (2). However, despite these findings, exercise remains a frontline treatment for retarding insulin resistance and the development of cardiovascular disease (4). The beneficial effects of exercise can outweigh any detrimental effects of excess glycogen accumulation in the diabetic heart postexercise.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
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