Fat Feeding Impairs Glycogen Synthase Activity in Mice Without Effects on Its Gene Expression

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To examine whether the effects of high-fat feeding on glycogen synthase (GS) activity and mRNA levels differ between diabetes-prone (C57BL/6J) and diabetes-resistant mice (NMRI), we measured GS activity and mRNA levels in muscle from C57BL/6J and NMRI mice fed a high-fat or normal chow diet for 3, 6, and 15months. As compared with chow feeding, fat feeding increased plasma insulin levels in C57BL/6J mice at 15 months (464 ± 29 *v* 267 ± 47 pmol/L, *P* = .005), which was associated with elevated plasma glucose levels at 15 months (5.3 ± 0.3 *v* 3.8 ± 0.2 mmol/L, *P* = .001). Fat feeding increased plasma insulin levels also in NMRI mice at 15 months (705 ± 145 *v* 275 ± 64 pmol/L, *P* = .01) without, however, a rise of plasma glucose levels. In parallel with increased insulin levels, decreased muscle GS fractional velocity (FV) was observed at 6 (49.0% ± 2.6% *v* 69.1% ± 7.3%, *P* = .04) and 15 (45.8% ± 1.8% *v* 53.4% ± 1.6 %, *P* < .01) months but not at 3 months in the fat-fed C57BL/6J mice. Similarly, there was a significant decrease in GS fractional activity at 3 (57.9% ± 4.3% *v* 70.4% ± 2.6 %, *P* < .03) and 15 (47.3% ± 2.4% *v* 56.4% ± 2.1%, *P* = .02) but not at 6 months in the fat-fed NMRI mice. The decrease in GS activity was not associated with changes in mRNA levels at any time points. We conclude that (1) fat feeding results in similar elevation of plasma insulin levels and impairs GS activity in C57BL/6J and NMRI mice, and (2) the changes in GS activity do not involve effects on gene expression.

IMPAIRMENT OF GLUCOSE metabolism in skeletal muscle is a characteristic feature of patients with type 2 diabetes and is mostly accounted for by impaired glycogen synthesis.[1,2] There have been several reports of impaired muscle glycogen synthase (GS) activity in first-degree relatives of patients with type 2 diabetes,[3-5] suggesting that genetic factors could play a role. An *Xba*I polymorphism in the skeletal muscle GS gene has been associated with type 2 diabetes and features of insulin resistance.[6] One explanation for the effect of this intronic variant has been ascribed to the incapacity of *Xba*I carriers to upregulate their GS protein in response to exercise.[7] Apart from this intronic single-nucleotide polymorphism, only rare alterations in the coding region of the GS gene have been found in type 2 diabetic patients.[8] In addition to exercise, glycogen synthesis is also sensitive to dietary manipulations, and high-fat feeding of mice and rats is known to induce insulin resistance, which includes changes in GS activity.[9-12] In C57BL/6J mice, genetic linkage between the phenotype of developing glucose intolerance in response to high-fat feeding and the GS region on chromosome 7 has been reported but it remains unclear whether the linkage really involves the GS gene.[13] How a high fat-diet influences the GS activity and whether this involves changes in gene expression as reported for the insulin receptor substrate-1 and phosphatidylinositol 3-kinase[14] is not known.

This study was therefore designed to examine whether (1) fat feeding results in impaired muscle GS activity through changes in gene expression, and (2) whether such changes would be more prominent in diabetes-prone C57BL/6J than in diabetic-resistant NMRI mice, which would suggest an inherited sensitivity of the GS gene to environmental triggers like a high-fat diet in the former.

MATERIALS AND METHODS

Animals and Diets

Female mice of the C57BL/6J and NMRI strains were obtained at 4 weeks of age from Bomholtgaard Breeding and Research Centre, Ry, Denmark. Eight to 10 mice from each strain received either a high-fat diet (Research Diets Inc, New Brunswick, NJ) or an ordinary rodent chow diet (Research Diets Inc) for 3 time periods of 3, 6, and 15 months. Animals were kept 4 to 5 per cage in a temperature-controlled (22 ± 1°C) room with a 12-hour light-dark cycle. The mice had free access to food and water throughout the study periods. On a caloric base, the high-fat diet consisted of 16.4% protein, 25.6% carbohydrates, and 58.0% fat (total, 23.4 kJ/g), and the chow diet consisted of 25.8% protein, 62.8% carbohydrates, and 11.4% fat (12.6 kJ/g). At the end of each study period, blood samples were taken at 11 AM, ie, 5 hours after lights on, from the intraorbital retrobulbar plexus for the measurement of plasma insulin and glucose. After the nonfasting mice were killed during anesthesia, gastrocnemius muscles were taken immediately into liquid nitrogen and stored at −80°C until analyzed. The study was approved by the Animal Ethics Committee at Lund University. Plasma glucose was determined with an automated glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA). Plasma insulin was measured using a double-antibody radio immunoassay (Linco, St Charles, MO).

GS Activity

Extraction of muscle samples and assays for GS were slightly modified from previously described methods.[4] Briefly, glycogen synthase activity was measured in the presence of a near physiological concentration of 0.17 mmol/L glucose-6-phosphate and in the presence of a high concentration of 7.2 mmol/L glucose-6-phosphate. The concentration of uridine diphosphate glucose (UDPG) was 0.14 mmol/L in the reaction. Glycogen synthase activity was expressed as nanomoles of

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Table 1. Body Weight, Plasma Glucose, and Insulin in C57BL/6J Mice Fed With a High-Fat or Chow Diet for Various Periods of Time

	3 Months		6 Months		15 Months	
	Fat Diet	Chow Diet	Fat Diet	Chow Diet	Fat Diet	Chow Diet
Body weight (g)	27.2 ± 0.9*	21.3 ± 0.5	32.2 ± 1.6*	25.0 ± 0.6	44.3 ± 1.6*	26.5 ± 0.8
Plasma glucose (mmol/L)	9.1 ± 0.6	7.8 ± 0.5	8.0 ± 0.4	7.5 ± 0.3	5.3 ± 0.3*	3.8 ± 0.2
Plasma insulin (pmol/L)	250.1 ± 37.4	211.2 ± 45.0	485.0 ± 76.1*	176.7 ± 26.6	463.5 ± 29.0*	266.5 ± 46.7

NOTE. Data are means ± SE (n = 8–10).
*$P < .01$ *v.* chow-fed mice at the same time point.

UDPG incorporated into glycogen per minute per milligram extract of protein. Fractional velocity (FV) was calculated as the ratio between GS activities at 0.17 mmol/L glucose-6-phosphate and 7.2 mmol/L glucose-6-phosphate. Total activity of GS refers to the activity in the presence of 7.2 mmol/L glucose-6-phosphate.

GS mRNA

The mRNA expression of GS was examined using a modified "primer-dropping" reverse-transcriptase polymerase chain reaction (RT-PCR) method.[15] Total RNA was isolated from the muscle biopsies by the acid guanidinium thiocyanate method,[16] and subjected to DNase I (Promega, Madison, WI) treatment according to manufacturer's instruction to avoid genomic DNA contamination. Four hundred nanograms treated total RNA of each sample was then reverse-transcribed in 40-μL reaction with a 5 μmol/L oligo$(dT)_{18}$ primer in the presence of 200 U of SUPERSCRIPT II Reverse Transcriptase (Life Technologies, Glasgow, Scotland) and 25 μmol/L dNTP for 60 minutes at 37°C according to the manufacturer's instruction. After heat-inactivation of the reverse-transcriptase at 95°C for 5 minutes, 2 μL of the RT reaction was added to 18 μL of PCR mixture containing 1x PCR buffer, 0.2 mmol/L dNTP, 5% dimethylsulfoxide, 0.5 U Taq polymerase, 0.2 μmol/L primers for the GS gene, and 0.2 μmol/L primers for the cyclophilin gene as the reference. The mouse GS primer pairs (from 5′ to 3′) are GCGCTACCTGTGTGCTGGCGC and GTTGAGCCGGGCCAATGCCTC. The mouse cyclophilin primer pairs are GCAGGTCCATCTACGGAGAG and GCTGTCCACAGTCGGAAATGG. The PCR was run for 29 cycles (94°C, 30 seconds; 62°C, 30 seconds; 72°C, 30 seconds) and followed by a final extension at 72°C for 10 minutes. The PCR condition was optimized according to the primer-dropping method[15] to maintain coamplification within the exponential phase. Selection of the cyclophilin gene as a reference was based on its unaffected expression in the insulin-resistant and diabetic state.[17] Validation of this method using RNase protection assay has been described in a previous study.[18] PCR products were separated on a 2% agarose gel containing ethidium bromide, photographed with UPP-110HA printing paper (Sony, Tokyo, Japan), and quantitated using Personal Densitometer SI scanner together with ImageQuant software (Molecular Dyanmics, Sunnyvale, CA). The mRNA signals were expressed relative to that of cyclophilin.

Statistical Analysis

Data are expressed as means ± SEM. Statistical analysis was performed using a NCSS 6.0.21 statistical package (NCSS Statistical Software, Kaysville, UT). The significance of difference within or between groups was tested by Wilcoxon or Mann-Whitney rank tests when appropriate.

RESULTS

Effect of Fat and Chow Feeding on Insulin and Glucose Levels and Body Weight in C57BL/6J and NMRI Mice

Plasma insulin levels in the fat-fed C57BL/6J mice did not differ at 3 months, but were higher at 6 ($P < .001$) and 15 ($P = .005$) months as compared with the chow-fed C57BL/6J mice (Table 1). Plasma insulin levels in the fat-fed NMRI mice were significantly higher at 3 months ($P = .01$), slightly higher at 6 months ($P = .20$), and again significantly higher at 15 months ($P = .01$) compared with the chow-fed C57BL/6J mice (Table 2). There was no significant difference in plasma insulin levels between C57BL/6J and NMRI mice under either fat- or chow-fed conditions. Plasma glucose levels in the fat-fed C57BL/6J mice were similar at 3 and 6 months, but significantly higher after 15 months ($P = .001$) compared with the chow-fed mice (Table 1). No significant elevation of glucose levels was seen in the fat-fed compared with the chow-fed NMRI mice at 3, 6, and 15 months (Table 2). Body weight of the fat-fed C57BL/6J mice was 28% higher at 3 months ($P < .001$), again 28% higher at 6 months ($P < .001$), and 69% higher at 15 months ($P < .001$) as compared with the chow-fed C57BL/6J mice (Table 1). However, body weight of the fat- and chow-fed NMRI mice was similar at 3 months, and only slightly higher at 6 ($P = .04$) and 15 ($P = .06$) months as compared with the chow-fed NMRI mice (Table 2). Body weight of C57BL/6J mice was significantly lower than that of NMRI mice under chow-fed conditions throughout the whole study period ($P < .01$). Under fat-fed conditions body weight of C57BL/6J mice

Table 2. Body Weight, Plasma Glucose, and Insulin in NMRI Mice Fed With a High-Fat or Chow Diet for Various Periods of Time

	3 Months		6 Months		15 Months	
	Fat Diet	Chow Diet	Fat Diet	Chow Diet	Fat Diet	Chow Diet
Body weight (g)	40.2 ± 2.8	37.2 ± 1.3	42.1 ± 2.6*	37.6 ± 0.9	48.0 ± 3.8	38.3 ± 1.7
Plasma glucose (mmol/L)	9.9 ± 0.2	9.5 ± 0.3	7.9 ± 0.3	7.7 ± 0.2	5.3 ± 0.2	5.5 ± 0.2
Plasma insulin (pmol/L)	401.4 ± 83.2*	170.8 ± 32.3	609.1 ± 232.1	254.6 ± 51.3	705.3 ± 144.7*	275.3 ± 64.4

NOTE. Data are means ± SE (n = 8–10).
*$P < .05$ *v.* chow-fed mice at the same time point.

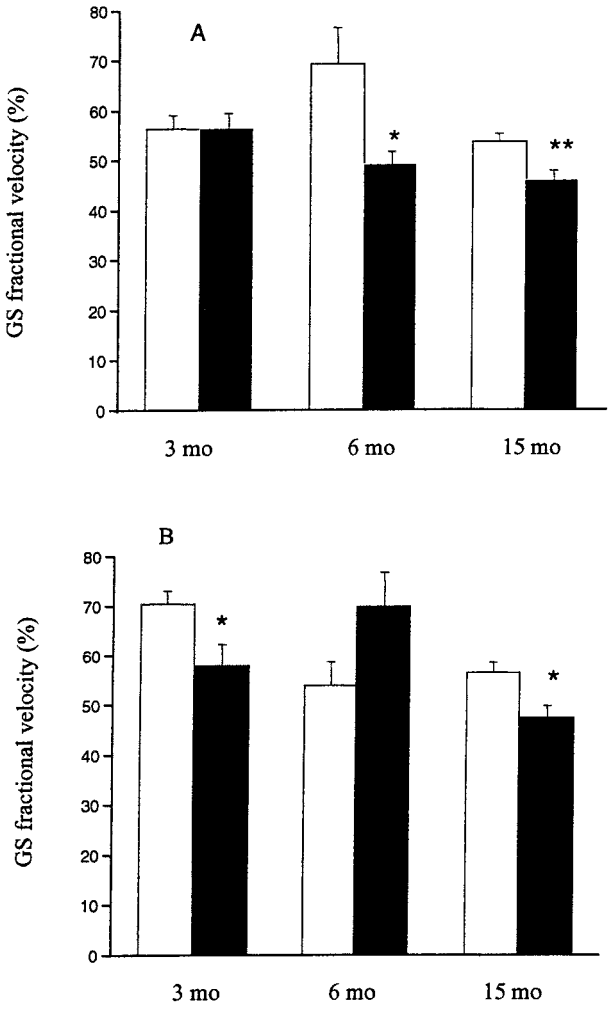

Fig 1. Influence of fat feeding on muscle GS FV in (A) C57BL/6J mice at 3 (56.4% ± 3.0% *v* 56.3% ± 2.7%, n = 9-10), 6 (49.0% ± 2.6% *v* 69.1% ± 7.3%, n = 8), and 15 months (45.8% ± 1.8% *v* 53.4% ± 1.6%, n = 10); and (B) in NMRI mice at 3 (57.9% ± 4.3% *v* 70.4% ± 2.6%, n = 8), 6 (69.8% ± 6.8% *v* 53.9% ± 4.7%, n = 8), and 15 months (47.3% ± 2.4% *v* 56.4% ± 2.1%, n = 10). **P* < .05, ***P* < .01 *v* chow-fed mice of the same strain at the same time point. (■) High-fat diet; (□) chow diet.

remained lower at 3 and 6 months ($P < .01$) but similar at 15 minths as compared with NMRI mice.

GS Activity

GS FV was similar at 3 months (56.4% ± 3.0% *v* 56.3% ± 2.7%, P = not significant [NS], n = 9-10), but lower at 6 (49.0% ± 2.6% *v* 69.1% ± 7.3%, $P = .04$, n = 8) and 15 (45.8% ± 1.8% *v* 53.4% ± 1.6%, $P < .01$, n = 10) months in the fat-fed compared with the chow-fed C57BL/6J mice (Fig 1A). No consistent correlation was observed between GS FV and plasma insulin or glucose levels in either fat-fed or chow-fed C57BL/6J mice at any time point. No change with time in GS FV was seen in the chow-fed C57BL/6J mice. In the fat-fed NMRI mice, GS FV was significantly lower at 3 (57.9% ± 4.3% *v* 70.4% ± 2.6%, $P < .03$, n = 8), slightly lower at 6 (69.8% ± 6.8% *v* 53.9% ± 4.7 %, $P = .10$, n = 8), and significantly lower at 15 (47.3% ± 2.4% *v* 56.4% ± 2.1%, $P = .02$, n = 10) months as compared with that of the chow-fed NMRI mice (Fig 1B). GS FV in the chow-fed NMRI mice was lower at 6 ($P = .01$) and 15 ($P < .01$) months compared with that at 3 months (Fig 1B). No consistent correlation was observed between GS FV and plasma insulin or glucose in either fat-fed or chow-fed NMRI mice at any given time point. GS total activity was similar in fat- and chow-fed C57BL/6J mice at 3 (8.8 ± 0.5, 8.5 ± 1.0 nmol · min^{-1} · mg^{-1}, P = NS), 6 (10.8 ± 0.9, 9.0 ± 0.8 nmol · min^{-1} · mg^{-1}, P = NS), and 15 (7.7 ± 0.6, 8.1 ± 0.8 nmol · min^{-1} · mg^{-1}, P = NS) months (Fig 2A). In the fat-fed NMRI mice GS total activity was significantly higher at 3 months (14.3 ± 1.0 *v* 8.1 ± 0.4 nmol · min^{-1} · mg^{-1}, $P < .001$), slightly higher at 6 months (13.0 ± 1.3 *v* 9.7 ± 0.7 nmol · min^{-1} · mg^{-1}, $P = .08$), but lower at 15 months (7.9 ± 0.7 *v* 10.5 ± 1.2 nmol · min^{-1} · mg^{-1}, $P < .03$),

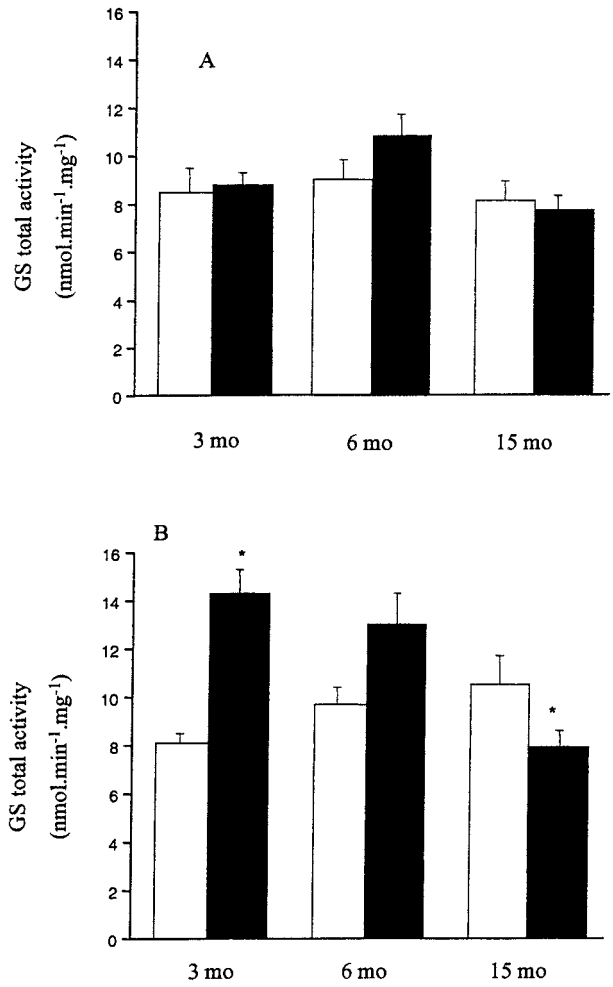

Fig 2. Influence of fat feeding on muscle GS total activity in (A) C57BL/6J mice at 3 (8.8 ± 0.5, 8.5 ± 1.0 nmol · min^{-1} · mg^{-1}, n = 9-10), 6 (10.8 ± 0.9, 9.0 ± 0.8 nmol · min^{-1} · mg^{-1}, n = 8), and 15 (7.7 ± 0.6, 8.1 ± 0.8 nmol · min^{-1} · mg^{-1}, n = 10) months; and (B) in NMRI mice at 3 (14.3 ± 1.0 *v* 8.1 ± 0.4 nmol · min^{-1} · mg^{-1}, n = 8), 6 (13.0 ± 1.3 *v* 9.7 ± 0.7 nmol · min^{-1} · mg^{-1}, n = 8), and 15 months (7.9 ± 0.7 *v* 10.5 ± 1.2 nmol · min^{-1} · mg^{-1}, n = 10). **P* < .05 *v* chow-fed mice of the same strain at the same time point. (■) High-fat diet; (□) chow diet.

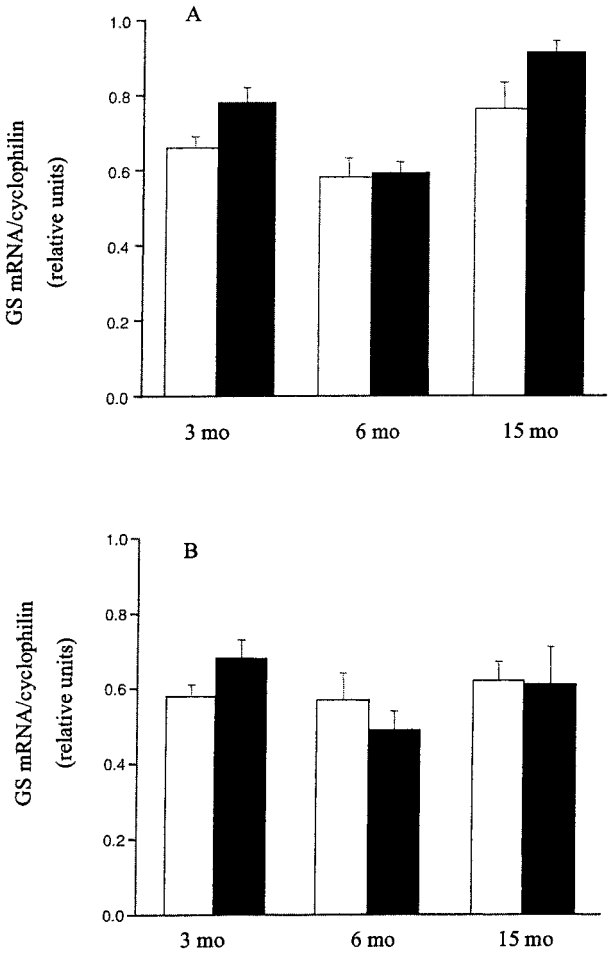

Fig 3. Muscle GS mRNA levels in (A) C57BL/6J mice and (B) NMRI mice fed with a high-fat diet or chow diet at 3, 6, and 15 months. (■) High-fat diet; (□) chow diet.

as compared with the chow-fed NMRI mice (Fig 2B). No consistent correlation was observed between GS total activity and plasma glucose or insulin values at any given time point in either fat- or chow-fed NMRI mice. No change with time in GS total activity was seen in either C57BL/6J or NMRI mice fed with a chow diet.

GS mRNA

Muscle GS mRNA levels were similar in fat- and chow-fed C57BL/6J mice at 3 (0.78 ± 0.04 *v* 0.66 ± 0.03 relative units, P = NS), 6 (0.59 ± 0.03 *v* 0.58 ± 0.05 relative units, P = NS), and 15 (0.91 ± 0.03 *v* 0.76 ± 0.07 relative units, P = NS) months (Fig 3A). No significant correlation between GS mRNA and FV was observed in C57BL/6J mice at any given time point. GS mRNA levels were also similar in the fat- and chow-fed NMRI mice at 3 (0.68 ± 0.05 *v* 0.58 ± 0.03), 6 (0.49 ± 0.05 *v* 0.57 ± 0.07), and 15 (0.61 ± 0.10 *v* 0.62 ± 0.05 relative units; P = NS for all) months (Fig 3B). No significant correlation was observed between GS mRNA levels and GS FV values at any given time point in either chow- or fat-fed NMRI mice.

DISCUSSION

In this study we examined the long-term influence of a high-fat diet on GS activity and GS gene expression in skeletal muscle of C57BL/6J and NMRI mice compared with a low-fat diet. As the diet was given for a period of 15 months it represents almost a life-long exposure of mice to the diet. The high-fat diet induced insulin resistance in both strains of mice, based on the clear elevation of plasma insulin levels and the decrease in muscle GS FV. In contrast, whereas high-fat–fed NMRI mice had similar glucose levels as chow-fed mice, the high-fat–fed C57BL/6J mice had higher glucose levels after 15 months on the diet than their corresponding controls. In accordance with the effect of fat feeding on GS FV shown in this study, Hedeskov et al reported decreased muscle GS activity in the NMRI mice on a 80% fat diet for only 3 months.[12] Partially in contrast with these findings, Seldin et al. reported increased muscle GS FV in C57BL/6J and unchanged GS FV in A/J mice on a 35% fat diet for 4 months.[13] These differences could be explained in part by the difference in dietary fat content (58% *v* 35%) and/or different mouse strains. As only the C57BL/6J mice had higher glucose levels after high-fat feeding compared to chow, this may not be attributable to fat-induced insulin resistance alone as both strains showed insulin resistance and impaired GS activity. In contrast, the elevation of glucose levels in fat-fed C57BL/6J mice could be attributed to relative insulin insufficiency, as impaired glucose-stimulated insulin secretion has been reported in fat-fed C57BL/6J mice.[19,20]

We also tested the hypothesis whether fat-induced decrease in muscle GS activity represents the consequence of changes in gene expression. This is unlikely as we did not observe any significant difference in GS mRNA levels at any give time point between fat- and chow-fed conditions in either the C57BL/6J or the NMRI strain. Thus, the fat-induced changes in GS FV and GS total activity most likely are due to post-trancriptional modification. Interestingly, Eldar-Finkelman et al reported a 2-fold increase in GS kinase-3 (GSK-3) activity in fat tissue of the C57BL/6J mice fed with a 35% fat diet for 4 months.[21] Although not measured in the current study, it is possible that increased GSK-3 activity could contribute to the decreased GS FV. In type 2 diabetic patients, impaired muscle GS activity can be seen in the presence of normal GS[22,23] but elevated GSK-3 protein levels.[24] It is unlikely that the changes in GS activity are the result of increased body weight as both strains showed the same impairment in GS activity but only the C57BL/6J mice gained weight. Changes in muscle GS activity seem to be reversible as improved muscle GS activity has been associated with improved insulin sensitivity after exercise in rats fed with a high-fat diet.[10] Also, treatment with low-fat and high-carbohydrate diet improves impaired insulin activation of muscle GS activity in type 2 diabetic patients.[25] Given the findings of normal and even recovered GS FV during the early and intermediate period of fat feeding, it is more likely that the lipid metabolites[26] rather than weight gain cause impaired muscle GS activity. In support of this, it has been reported that increased muscle triglyceride and long-chain fatty acyl-coenzyme A (CoA) are associated with insulin resistance in fat-fed rats,[27] and increased long-chain fatty acyl-CoA inhibits GS.[28]

Although our data at first glance question a role of inherited defects in the GS enzyme as cause of insulin resistance and

susceptibility to diabetes in the C57BL/6J mice, there were some interesting differences between the C57BL/6J and NMRI strains with respect to the GS enzyme. In the NMRI mice, total GS activity was significantly increased after 3 months of fat feeding compared with chow-feeding and a similar trend was seen even after 6 months. These data could reflect a compensatory increase in protein levels to overcome the decrease in enzyme activity in the NMRI mice, which was lacking in the C57BL/6J mice. After 15 months, total GS activity was decreased rather than increased in the NMRI mice, suggesting decompensation with aging. Interestingly, upregulation of GS protein levels was also seen in human subjects during prolonged exercise[7] and this capacity was lacking in human patients with the A2 allele of the *Xba*I polymorphism in the muscle GS gene, which is known to increase susceptibility to type 2 diabetes.[6] Therefore, the lack of increase in total GS enzyme activity, which reflects protein levels,[23,29] might still reflect an inherited defect in the enzyme in the C57BL/6J mice.

We conclude that fat feeding induces insulin resistance and impaired GS activity in C57BL/6J and NMRI mice, but that the changes in GS activity do not involve effects on gene expression.

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