Resistance to Diet-Induced Obesity and Improved Insulin Sensitivity in Mice With a Regulator of G Protein Signaling–Insensitive G184S Gnai2 Allele

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OBJECTIVE—Guanine nucleotide binding protein (G protein)–mediated signaling plays major roles in endocrine/metabolic function. Regulators of G protein signaling (RGSs, or RGS proteins) are responsible for the subsecond turn off of G protein signaling and are inhibitors of signal transduction in vitro, but the physiological function of RGS proteins remains poorly defined in part because of functional redundancy.

RESEARCH DESIGN AND METHODS—We explore the role of RGS proteins and $G\alpha_{i2}$ in the physiologic regulation of body weight and glucose homeostasis by studying genomic "knock-in" mice expressing RGS-insensitive $G\alpha_{i2}$ with a G184S mutation that blocks RGS protein binding and GTPase acceleration.

RESULTS—Homozygous $G\alpha_{i2}^{G184S}$ knock-in mice show slightly reduced adiposity. On a high-fat diet, male $G\alpha_{i2}^{G184S}$ mice are resistant to weight gain, have decreased body fat, and are protected from insulin resistance. This appears to be a result of increased energy expenditure. Both male and female $G\alpha_{i2}^{G184S}$ mice on a high-fat diet also exhibit enhanced insulin sensitivity and increased glucose tolerance despite females having similar weight gain and adiposity compared with wild-type female mice.

CONCLUSIONS—RGS proteins and $G\alpha_{i2}$ signaling play important roles in the control of insulin sensitivity and glucose metabolism. Identification of the specific RGS proteins involved might permit their consideration as potential therapeutic targets for obesity-related insulin resistance and type 2 diabetes. ***Diabetes*** **57:77–85, 2008**

Obesity is reaching epidemic proportions worldwide (1). In the U.S., obesity is the most common chronic disease, affecting more than one in four of all Americans, including children, and its incidence has been steadily increasing for the past 20 years (2). Obesity is a major risk factor for the development of insulin resistance and type 2 diabetes and is associated with hypertension and atherosclerosis (3). A better understanding of the biological, genetic, and molecular mechanisms involved in obesity and diabetes is crucial for the development of new therapeutic strategies or antidiabetes drugs (4).

Obesity develops when energy intake exceeds energy expenditure. Regulation of food intake and energy expenditure is mainly controlled by neurons in the hypothalamus and brainstem with the help of several neuropeptides and neurotransmitters responding to long-term adipostatic signals (leptin and insulin) and to acute satiety/hunger hormones (ghrelin) (5). Many key receptors that mediate effects of neuropeptides and neurotransmitters involved in central regulation of energy balance are G protein–coupled receptors (GPCRs) (6). More than 40 GPCRs have been implicated in regulation of body weight through mouse knockout and transgenic models, quantitative trait loci from crossbreeding experiments, and linkage studies (6,7). The GPCRs involved in control of body weight and carbohydrate and lipid homeostasis are active targets in drug development. These include the melanocortin system (8); neuropeptide Y receptors (9); the cannabinoid system (10); monoamine GPCRs, including serotonin, adrenergic, and histamine receptors (11); the nicotinic acid receptor (12); and gut hormone systems, including ghrelin and the glucagon-like peptide-1 receptor (13).

Most GPCRs signal through one or more G protein, including those of Gαi, Gαs, and Gαq family members, to effectors such as adenylyl cyclases, phospholipase C, ion channels, and cGMP phosphodiesterase (14). Hormone-activated GPCRs promote guanine nucleotide exchange on the Gα subunit, and then activated $G\alpha^{GTP}$ and Gβγ subunits dissociate to regulate their downstream effectors and cellular responses. GTP hydrolysis by Gα restores the inactive heterotrimeric $G\alpha^{GDP}\beta\gamma$. Regulators of G protein signaling (RGSs, or RGS proteins) are a large and diverse family initially identified as GTPase-activating proteins of heterotrimeric G protein Gα subunits. They serve mostly negative modulatory roles in G protein–mediated signal transduction (15). Mechanistically, RGSs regulate GPCR responses by binding to and stimulating the GTPase activity of the receptor-activated GTP-bound Gα subunits to rapidly deactivate Gα (16). RGS proteins also can regulate G protein–effector interactions in other ways, either by competitively inhibiting Gα binding to effectors such as phospholipase C or by serving as a protein adaptor that can recruit diverse effectors or regulators to the activated G proteins (17–19). To date, 37 human genes that encode proteins containing an RGS or RGS-like domain have been identified and, most currently, known RGS proteins regulate Gi and Gq signaling (20). The activity, subcellular distribution, and expression levels of RGS

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DEXA, dual-energy X-ray absorptiometry; GPCR, G protein–coupled receptor; RGS, regulator of G protein signaling.

proteins are dynamically regulated throughout development and in response to pharmacological or pathological alterations in cell signaling (21). Furthermore, there is emerging interest in RGS proteins as drug targets (18, 22,23). However, the physiological functions of RGS proteins remain poorly defined. Little information is available implicating RGS proteins in control of metabolic activity to maintain body weight and energy balance (24,25).

There are more than 15 RGS proteins that can act on Gi/o family members (18). To overcome this redundancy of RGS function and to reveal the total contribution of RGS proteins in the regulation of any given G protein, we used genomic knock-in animals expressing an RGS-insensitive allele, G184S, of $G\alpha_{i2}$ (26). The only known effect of the G184S mutation in Gα is to disrupt RGS binding, thus preventing GTPase-activating protein activity (27,28). The knock-in approach maintains the normal distribution and level of $G\alpha_{i2}$ expression (26). As described previously, homozygous $G\alpha_{i2}^{G184S}$ mice display a complex phenotype that reveals a substantial role for RGS proteins acting on $G\alpha_{i2}$ (26,29). They have enhanced signaling through $G\alpha_{i2}$, alterations in cardiac chronotropy and myelomonocytic cell numbers, and short stature. Based on preliminary observations that the mice were lean, we evaluate, in the present study, the influence of endogenous RGS protein action on $G\alpha_{i2}$ on fat deposits and glucose metabolism by placing RGS-insensitive $G\alpha_{i2}$ mice ($G\alpha_{i2}^{G184S}$ homozygotes) on high-fat or low-fat diets. Male $G\alpha_{i2}^{G184S}$ mice are resistant to high fat–induced weight gain, demonstrate significantly decreased body fat, and are protected from insulin resistance. This appears to be a result of increased energy expenditure and is accompanied by enhanced peripheral insulin sensitivity. Interestingly, female $G\alpha_{i2}^{G184S}$ mice also exhibit enhanced insulin sensitivity and increased glucose tolerance despite weight gain and adiposity that is similar to wild-type females on the high-fat diet. These findings demonstrate that RGS proteins and $G\alpha_{i2}$ signaling play a critical role in the regulation of insulin sensitivity and glucose homeostasis in vivo. Furthermore, RGS proteins might provide new therapeutic opportunities for pharmacological prevention of high-fat diet–induced obesity and type 2 diabetes.

RESEARCH DESIGN AND METHODS

All protocols and procedures were approved by the university committee on use and care of animals, and animal care was overseen by the unit for laboratory animal medicine (University of Michigan). The homozygous $G\alpha_{i2}^{G184S/G184S}$ mice (hereafter called $G\alpha_{i2}^{G184S}$) were previously described (26). Mice were originally obtained on a hybrid 129SVJ/C57Bl/6 background and were subsequently backcrossed at least five generations onto the C57BL/6J strain. Age- and sex-matched littermates were used for all experiments.

Female and male mice (wild-type and homozygous $G\alpha_{i2}^{G184S}$ mice) were maintained on a 12-h light/12-h dark schedule and fed standard laboratory chow and water ad libitum. Mutant mice shared the same cage with their littermate controls. At 4 weeks of age, mice were randomly allocated to either a low-fat diet (10% fat, 70% carbohydrate, and 20% protein; D12450B; Research Diets, New Brunswick, NJ) or a high-fat diet (45% fat, 35% carbohydrate, and 20% protein, 4.7 kcal/g; D12451; Research Diets) for 25 weeks.

Body weight was measured every other week. Food intake was measured at 10–11 weeks. Each mouse was housed in an individual cage, and food was weighed daily for 7 consecutive days. Energy expenditure was measured at 11 weeks and body composition (dual-energy X-ray absorptiometry [DEXA]) at 16 weeks. Glucose tolerance tests were performed at 16 and 25 weeks. Mice were killed at 26 weeks following 5 h of food deprivation, serum was frozen, and tissues, including four different fat depots, were removed, weighed, and immediately frozen in liquid nitrogen for storage at −70°C or were fixed in 10% neutral-buffered formalin for histological analysis.

Blood analysis. Glucose levels were determined in whole blood from mouse tails using an Ascensis ELITE XL blood glucose meter (Bayer, Mishawaka, IN).

TABLE 1
Metabolic phenotyping of female homozygous $G\alpha_{i2}^{G184S/G184S}$ mice on standard diet

	Wild type	$G\alpha_{i2}^{GS/GS}$
n	9	10
Body weight (g)	24.5 ± 0.7	19.7 ± 0.5*
Serum glucose (mg/dl)		
Overnight fasting	96 ± 5	107 ± 11
Nonfasting	153 ± 15	136 ± 8
Insulin (ng/ml)	0.23 ± 0.03	0.17 ± 0.02
Fat mass (%)	17.1 ± 0.8	15.3 ± 0.8
Plasma leptin (ng/ml)	4.3 ± 0.6	2.9 ± 0.6

Data are means ± SE. Female mice were given standard diet for 20–22 weeks, after which weight, glucose (fasting or nonfasting), insulin (nonfasting), and leptin were measured. Fat mass was estimated by DEXA. *$P < 0.0001$ by *t* test.

Serum insulin and leptin were determined by enzyme-linked immunosorbent assay (CrystalChem, Downers Grove, IL).

Energy expenditure. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were simultaneously measured by indirect calorimetry using the Oxymax System (Columbus Instruments, Columbus, OH). Mice were housed in separate chambers with free access to food and water for 3 days for acclimatization before starting the measurements. Oxygen consumption was measured at 20-min intervals for a total of 20 h and was normalized to body weight. Data during the light and dark cycles were calculated separately.

Body fat analysis. Animals were anesthetized with isoflurane (5% for induction and 1–2% for maintenance), placed in the prone position, and scanned using DEXA on a Mouse Densitometer (PIXImus; GE Medical Systems). Body composition was estimated with pDEXA SABRE software. Measurements included total mass, total fat mass, total lean mass, and body fat percentage.

Glucose tolerance tests. Mice were fasted overnight for 14 h with free access to water. Each mouse then received an intraperitoneal injection of glucose (2 mg/g body wt). Blood samples were taken from the tail vein before glucose injection and at 15, 30, 60, 120 min after the injection, and whole blood glucose was determined.

Insulin tolerance test. To measure whole-body insulin sensitivity, insulin tolerance tests were applied to 18- to 20-week-old female mice after 5 weeks of high-fat diet challenge. Mice were fasted for 4 h with free access to water in the morning and then injected intraperitoneally with regular insulin (0.5 units/kg body wt; Sigma). Tail-blood samples were obtained before insulin administration (time-zero sample), as well as at 15, 30, 60 and 120 min after the insulin injection, and whole blood glucose was determined.

Liver triglyceride measurement. Total liver lipids were extracted according to a method modified from that of Folch et al. (30). Briefly, snap-frozen liver tissues (70 mg) were homogenized in 1.5 ml chloroform:methanol (2:1 vol/vol) solution and incubated at room temperature with shaking for 4 h, and then 0.75 ml of 0.1 mol/l NaCl was added into the liver tissue homogenate. The organic phase was collected, dried, and resuspended in 0.2 ml of 3 mol/l KOH followed by incubating at 70°C for 1 h to hydrolyze the triglycerides. Then, 0.6 ml of 1 mol/l $MgCl_2$ was added. Glycerol was measured using the Free Glycerol Reagent (Sigma). The amount of triglycerides was calculated by conversion from the glycerol content.

Statistical analyses. Comparisons of individual-group means used a two-tailed Student's *t* test. To compare multiple datasets, a two-way ANOVA with Bonferroni posttest was used. All statistical calculations were done using GraphPad Prism version 4 (GraphPad Software, San Diego, CA).

RESULTS

Adult $G\alpha_{i2}^{G184S}$ mice have lower body weight and display subtle metabolic alterations on low-fat or standard diets. The most apparent physiological phenotype of the male and female homozygous RGS-insensitive $G\alpha_{i2}^{G184S}$ mice was their small size and considerably reduced body weight (Table 1) (26). Early phenotyping studies on a mixed 129xC57/Bl/6J background showed individual mutant mice with dramatically reduced fat stores. However, after backcrossing onto the C57/Bl/6J background, the metabolic phenotype was significantly blunted. Female 20- to 22-week-old $G\alpha_{i2}^{G184S}$ mutant mice

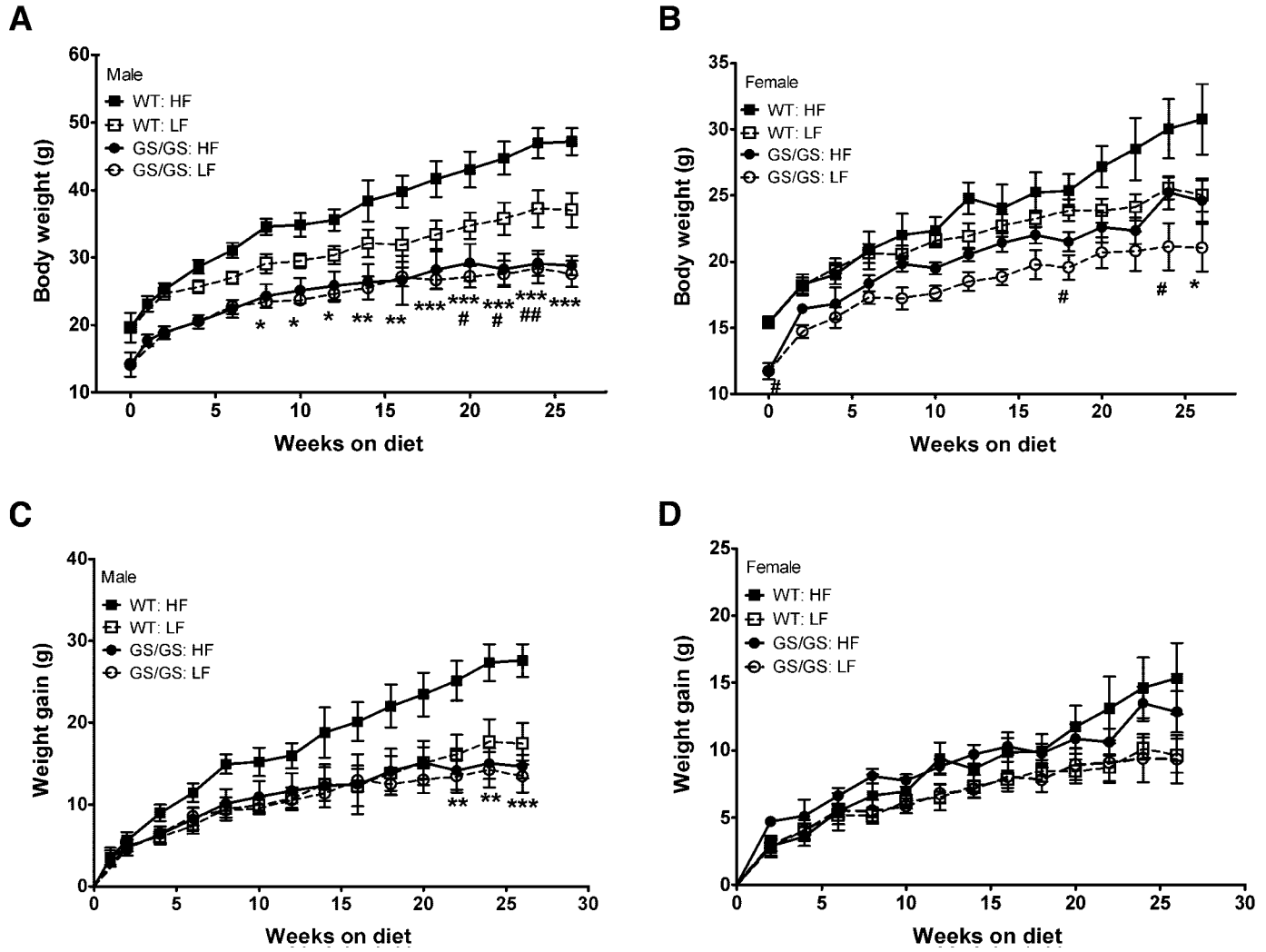

FIG. 1. Decreased body weight in $G\alpha_{i2}^{G184S}$ homozygous males during the feeding study. *A* and *B*: Body weight in male and female. $G\alpha_{i2}^{G184S}$ homozygous (GS/GS) mice have significantly lower body weight than wild-type (WT) mice during both high-fat (HF) or low-fat (LF) feeding periods. Genotype effect: $P < 0.0001$ by two-way ANOVA. *C* and *D*: Weight gains in male and female. Comparing wild-type and GS/GS mice on a high-fat diet: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by two-way ANOVA with Bonferroni posttest. Comparing wild-type and GS/GS mice on a low-fat diet: #$P < 0.05$, ##$P < 0.01$ by two-way ANOVA with Bonferroni posttest. Data are means ± SE; $n = 5$ mice/group.

fed a standard diet showed only a nonsignificant trend toward decreased body fat composition, as estimated with DEXA (Table 1). Consistent with reduced fat stores, they also show slightly decreased serum leptin levels. Similar nonsignificant changes were seen in males (see below). In addition, both male and female $G\alpha_{i2}^{G184S}$ mutants fed a standard or a low-fat diet also had a tendency toward decreased nonfasting serum insulin levels (Fig. S1), but they had minimal differences in glucose tolerance compared with wild-type animals. Thus, to elicit a latent phenotype, the mice were given a high-fat diet.

$G\alpha_{i2}^{G184S}$ males are protected from diet-induced obesity. High-fat feeding has been demonstrated to induce body weight gain and obesity and is associated with insulin resistance. We analyzed the effect of high- or low-fat diet on $G\alpha_{i2}^{G184S}$ homozygous and littermate control mice for 25 weeks. Control mice on either diet had higher body weights than mutants during the entire diet period (Fig. 1*A* for male and *B* for female mice); therefore, initial body weight was subtracted to determine weight gain (Fig. 1*C* and *D*). As expected, wild-type male mice showed a rapid weight gain on high-fat compared with low-fat diet. Surprisingly, mutant male mice fed the high-fat diet did not gain any more weight than those on the low-fat diet (Fig. 1*C*). The difference in weight gain between male wild-type and $G\alpha_{i2}^{G184S}$ mice on a high-fat diet was highly significant (Fig. 1*C*, weeks 22–26), but weight gains for male wild-type and $G\alpha_{i2}^{G184S}$ mice on the low-fat diet were comparable. In contrast to the males, female $G\alpha_{i2}^{G184S}$ mice exhibited similar weight gains on both diets compared with wild-type females (Fig. 1*D*).

Reduced body fat mass and hepatic triglyceride in male $G\alpha_{i2}^{G184S}$ mice. We sought to determine whether the reduced weight gain in male mutant mice was associated with alterations in adiposity. At necropsy, male $G\alpha_{i2}^{G184S}$ mice showed substantial reductions in the size of fat pads compared with high fat–fed wild-type mice (Fig. 2*A*). Body composition analysis by DEXA showed an ~30% reduction in percent body fat in mutant males compared with control mice after 16 weeks of high-fat diet ($P < 0.05$, Fig. 2*B*); however, no significant difference was observed in lean mass (data not shown). Consistent with the reduced adipose stores, there is a dramatically decreased leptin level in high fat–fed male $G\alpha_{i2}^{G184S}$ mice ($P < 0.001$, Fig. 2*C*). Females showed minor, nonsignificant changes in adiposity and a trend toward decreased

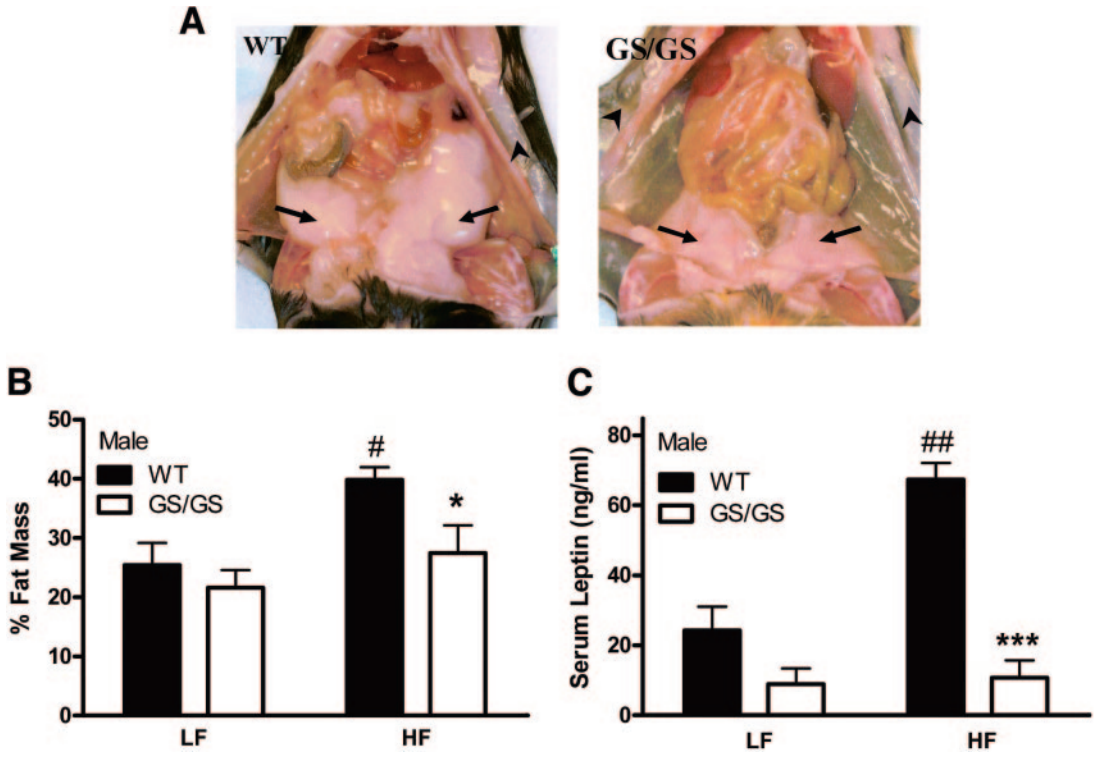

FIG. 2. Decreased adiposity in male $G\alpha_{i2}^{G184S}$ homozygote (GS/GS). *A*: Exposed ventral view of male mice on a 25-week high-fat diet (arrows, epididymal fat pads; arrow heads, dorsolumber fat pads). *B*. Fat mass calculated by DEXA after 16 weeks on a high-fat (HF) or low-fat (LF) diet. *C*: Five-hour fasting leptin concentration after a 25-week diet challenge. #$P < 0.05$, ##$P < 0.01$ vs. low-fat diet by t test; *$P < 0.05$, ***$P < 0.001$ vs. wild-type (WT) mice by t test. Data are means ± SE; $n = 5$ mice/group.

leptin levels but no significant effect of genotype on either parameter (Fig. S2). Furthermore, at the end of the diet study, high fat–fed mutant male mice showed markedly reduced white adipose tissue mass, including inguinal and perirenal fat ($P < 0.05$, Table 2). In contrast, brown adipose tissue (interscapular fat) and most other tissues (such as kidney) retained their normal proportion to body weight (Table 2). Interestingly, male mutant mice fed the low-fat diet also showed less epididymal fat than controls ($P < 0.05$, Table 2) but no significant difference in other fat tissues.

In addition, male mutant mice have significantly lower liver weights and liver triglyceride levels than wild-type mice (Fig. 3*C*). Furthermore, liver histology in control mice (Fig. 3*A* and *B*) exhibited vacuoles indicating increased hepatic lipid accumulation after 25 weeks of high-fat diet, whereas mutant mice had no appearance of vacuoles.

Increased metabolic rate relative to body weight in male $G\alpha_{i2}^{G184S}$ mice. We further investigated whether the resistance to diet-induced obesity observed in male mutant mice resulted from decreased food intake or increased energy expenditure. Food intake was measured for a continuous 7-day period for each group of mice after 10 weeks on a low- or high-fat diet. Food intake normalized to body weight was similar in the $G\alpha_{i2}^{G184S}$ and wild-type mice on a high- and low-fat diet (Figs. 4*A* and S3*A*). Indeed, energy consumption per gram of body weight was slightly, but not significantly, higher in both male and female mutant mice; therefore, reduced caloric intake alone does not appear to explain the reduced weight gain in males on a high-fat diet. After 11 weeks on a

TABLE 2
Weights of adipose tissue and other organs normalized to body weight in male $G\alpha_{i2}^{G184S/G184S}$ and control mice after a 25-week diet challenge

	Male low fat		Male high fat	
	Wild type	GS/GS	Wild type	GS/GS
White adipose tissue				
Epididymal	42.4 ± 5.4	28.8 ± 7.8*	43.7 ± 6.4	33.2 ± 7.3
Inguinal	25.7 ± 3.6	21.7 ± 6.8	43.4 ± 2.2	28.1 ± 5.9*
Perirenal	19.5 ± 2.7	11.8 ± 3.9	32.4 ± 1.3	17.5 ± 2.9*
Brown adipose tissue	13 ± 1.4	10.4 ± 2.1	15.3 ± 0.7	11.1 ± 1.4
Pancreas	7.9 ± 0.5	7.4 ± 0.4	7.2 ± 0.5	8.8 ± 1.2
Kidney	10.8 ± 0.6	11.7 ± 0.5	10.4 ± 0.7	11.7 ± 1.1
Lung	4.6 ± 0.3	5.3 ± 0.5	3.6 ± 0.1	4.9 ± 0.2

Data are means ± SE. Normalized fat pads and organ weights are percentages of body weight (milligrams per gram). Male wild-type and homozygous ($G\alpha_{i2}^{G184S/G184S}$ [GS/GS]) mice ($n = 5$ mice/group) were killed after 25-week diet challenges. Significant differences compared with wild-type mice: *$P < 0.05$ by two-way ANOVA with Bonferroni posttest.

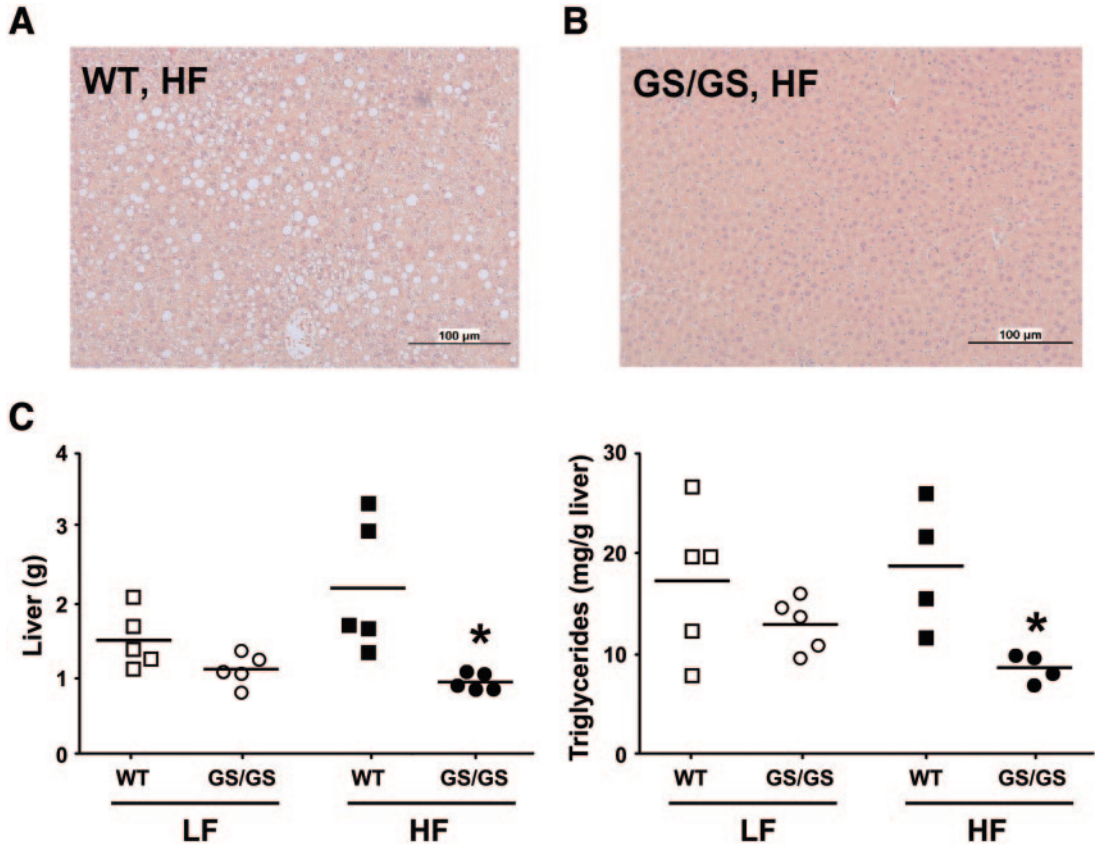

FIG. 3. Reduced hepatic steatosis in male $G\alpha_{i2}^{G184S}$ homozygotes (GS/GS). *A* and *B*: Histological analysis of the livers in male $G\alpha_{i2}^{G184S}$ homozygote and control mice. Representative hematoxylin and eosin–stained liver sections from wild-type (WT) (*A*) and $G\alpha_{i2}^{G184S}$ homozygote (GS/GS) (*B*) mice after 25 weeks on a high-fat (HF) diet challenge. *C*: Liver weight and hepatic triglycerides. *$P < 0.05$ by *t* test. Data are means and the actual scatter; $n = 4$–5 mice/group. LF, low fat.

high-fat diet, male $G\alpha_{i2}^{G184S}$ mice exhibited significantly higher rates of total and nighttime O_2 consumption per gram body weight than wild-type mice (Fig. 4*B* and *C*). While absolute O_2 consumption was reduced (to 83% of body weight) because of the smaller stature of the mutant mice, the absolute food intake (77% of body weight) was reduced even more than O_2 consumption, leading to a relatively higher use of energy compared with intake of energy. In addition to the greater nighttime V_{O_2}, there was also a small, but not statistically significant, increase in V_{O_2} in the light period that could represent either changes in basal metabolic rate or in daytime activity. In contrast, O_2 consumption in the female mutant mice (normalized to body weight) was not different from wild-type mice on either a low- or high-fat diet (Fig. S3*B* and *C*), consistent with their equivalent weight gain on high-fat diet. Males on a low-fat diet also did not show any increase in V_{O_2} (Fig. S4). All mice (male/female, low fat/high fat) showed a normal diurnal pattern with higher V_{O_2} during the night (active state) and lower rates during the day (resting state). The increased O_2 consumption in males may be related in part to our previously reported finding of increased activity in a telemetry study (26). Measurements of urine catecholamines revealed a modest increase in norepinephrine excretion in G184S male, but not female, mice on standard diet (Fig. S5).

Improved whole-body insulin sensitivity in $G\alpha_{i2}^{G184S}$ mice. The effect of high-fat diet on insulin levels and glucose tolerance was assessed in $G\alpha_{i2}^{G184S}$ and littermate control mice. Blood glucose and insulin levels were measured after 5 h of fasting. On the low-fat diet, $G\alpha_{i2}^{G184S}$ mice (male and female) showed slightly, but not significantly, reduced fasting plasma insulin levels compared with wild-type mice (Fig. S1 and Table 1). After 25 weeks of high-fat feeding, both male and female $G\alpha_{i2}^{G184S}$ mice showed markedly lower fasting insulin levels than control mice (Fig. 5*B*). Fasting blood glucose concentrations did not differ significantly despite the lower insulin levels in the mutants (Fig. 5*A*). To investigate whether the changes in plasma insulin levels upon high-fat feeding were associated with improved glucose handling, glucose tolerance tests were performed by intraperitoneal administration of 2 mg glucose/g body wt. As shown in Fig. 5, both male and female $G\alpha_{i2}^{G184S}$ mice on the high-fat diet were more efficient in clearing a bolus of glucose than control mice. Two-way ANOVA showed a highly significant genotype effect with significant decreases in glucose at 60 and 120 min for males and 60 min for females. The areas under the curve for glucose levels were reduced 28% in males and 22% in females (Fig. S6). Taken together, the low insulin, normal glucose, and improved glucose tolerance displayed by the $G\alpha_{i2}^{G184S}$ mice maintained on the high-fat diet indicate significantly enhanced insulin sensitivity. While an enhanced insulin sensitivity is expected in $G\alpha_{i2}^{G184S}$ males, in which weight gain and adiposity were significantly reduced, it was unexpected in females. Furthermore, this enhanced insulin sensitivity in female $G\alpha_{i2}^{G184S}$ mice was confirmed in insulin tolerance tests after only a 5-week high-fat diet challenge (Fig. 5*E*). Blood glucose levels in female mutant mice fell by 50% over a 1-hour period following an intraperitoneal injection of insulin (0.5 unit/kg body wt) but dropped by only ~25% in wild-type

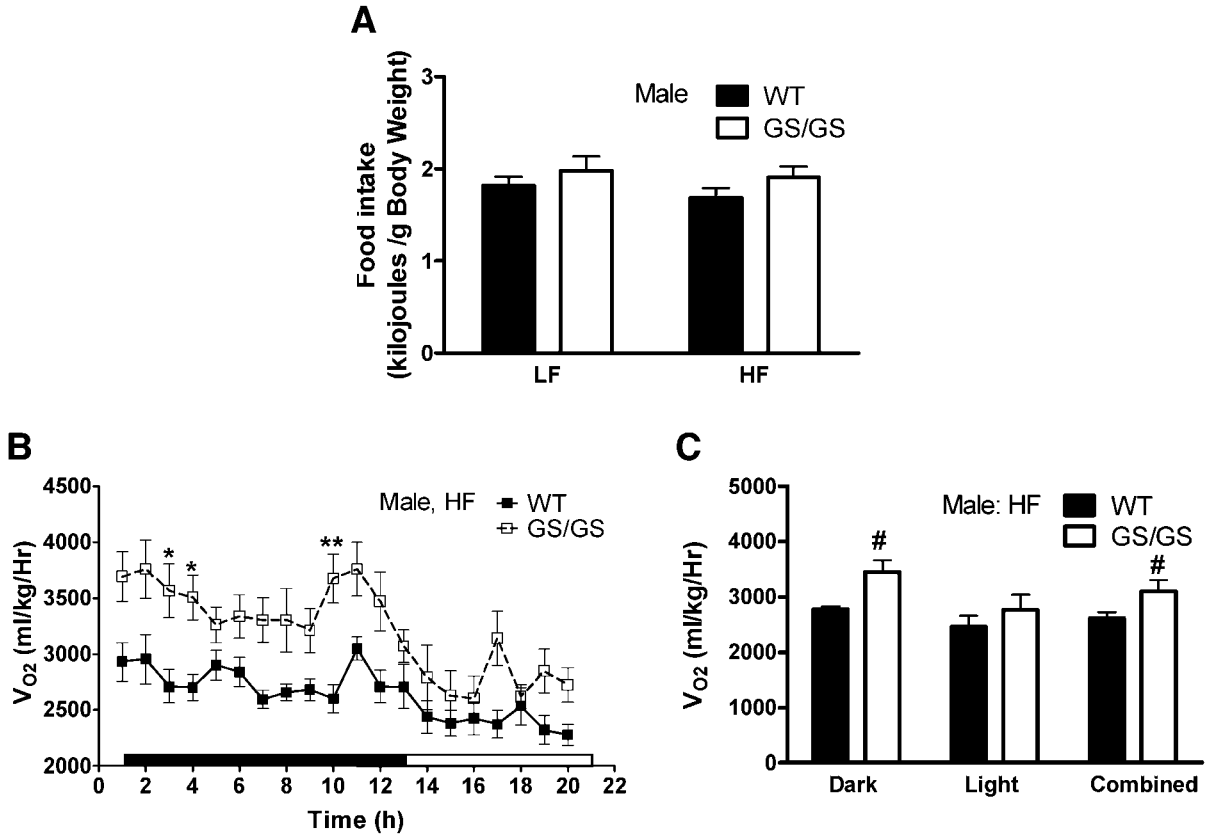

FIG. 4. Energy homeostasis of male wild-type (WT) and $G\alpha_{i2}^{G184S}$ homozygote (GS/GS) fed a high-fat (HF) or low-fat (LF) diet. *A*: Daily food intake measured over 7 days. *B* and *C*: Time course and summary of total oxygen consumption rate (Vo_2) measured during the dark and light periods. Genotype effect: $P < 0.0001$ by two-way ANOVA. *$P < 0.05$, **$P < 0.01$ by two-way ANOVA with Bonferroni posttest; significant differences at specific times are marked. #$P < 0.05$ by t test. Data are means ± SE; $n = 5$ mice/group.

mice (Figs. 5*E* and S6*C*). The reciprocal of the area under the curve was 21% greater in the $G\alpha_{i2}^{G184S}$ mutants than in wild-type females, consistent with an increase in insulin sensitivity.

DISCUSSION

RGS proteins speed the turn off of G protein signals and inhibit G_i- and G_q-mediated signal transduction, but the in vivo roles of RGS proteins remain poorly defined. Given the important role of G protein signaling, the goal of this study was to analyze the metabolic alterations of RGS-insensitive $G\alpha_{i2}^{G184S}$ mice to reveal the total contribution of RGS regulation at the $G\alpha_{i2}$ subunit in the regulation of body weight and glucose homeostasis. Male $G\alpha_{i2}^{G184S}$ homozygous mice are resistant to high fat–induced weight gain, have significantly decreased body fat due to increased energy expenditure, and show enhanced insulin sensitivity. Interestingly, female $G\alpha_{i2}^{G184S}$ homozygous mice also exhibit increased glucose tolerance and enhanced insulin sensitivity despite having a similar weight gain and adiposity compared with wild-type mice on a high-fat diet. These alterations were only revealed after an adipogenic dietary challenge because the RGS-insensitive mutation in $G\alpha_{i2}$ had minimal effects on fat mass or glucose metabolism in adult mice fed a standard or low-fat diet. The RGS-insensitive $G\alpha_{i2}^{G184S}$ mice should have enhanced, but receptor-dependent, G_{i2} activity in any tissue with functional RGS activity; thus, the adipogenic diet may activate signals that stimulate some important $G\alpha_{i2}$-coupled receptors to prevent the weight gain and improve glucose tolerance.

$G\alpha_{i2}$ expression is ubiquitous in peripheral tissues and is also present in many brain regions, including hypothalamus. At this point, we do not know whether the primary locus of effects seen in this model are central or peripheral, but G proteins clearly play important central roles both in general behavioral functions and in central control of metabolic processes mediated by a large number of GPCRs (6). Fat deposits are significantly reduced in male $G\alpha_{i2}^{G184S}$ mice on the high-fat diet, and this is associated with elevated energy expenditure. Because $G\alpha_{i2}^{G184S}$ mice on a low-fat diet do not show significantly increased Vo_2, it appears that the adipogenic diet somehow leads to enhanced signaling though one or more G_i-coupled receptor. This could be due to increased physical activity or central control of energy use. It is well known that lean animals generally exhibit an increase in insulin sensitivity, so it is not surprising that the male mice showed enhanced insulin sensitivity. However, female $G\alpha_{i2}^{G184S}$ mice also exhibited enhanced insulin sensitivity despite having weight gain and adiposity similar to wild-type female mice on the high-fat diet. While it is not clear why we see sex differences, it is commonly observed that males and females respond differently to high-fat diets, and a recent study also found sex differences in regulation of the neuropeptide Y signaling system in the hypothalamus (31). Regardless, it is likely that the increased insulin sensitivity displayed by the $G\alpha_{i2}^{G184S}$ mice includes a significant role of $G\alpha_{i2}$-mediated signaling in regulating glucose use or other metabolic functions and is not simply due to a reduction in body fat mass. This observation may explain

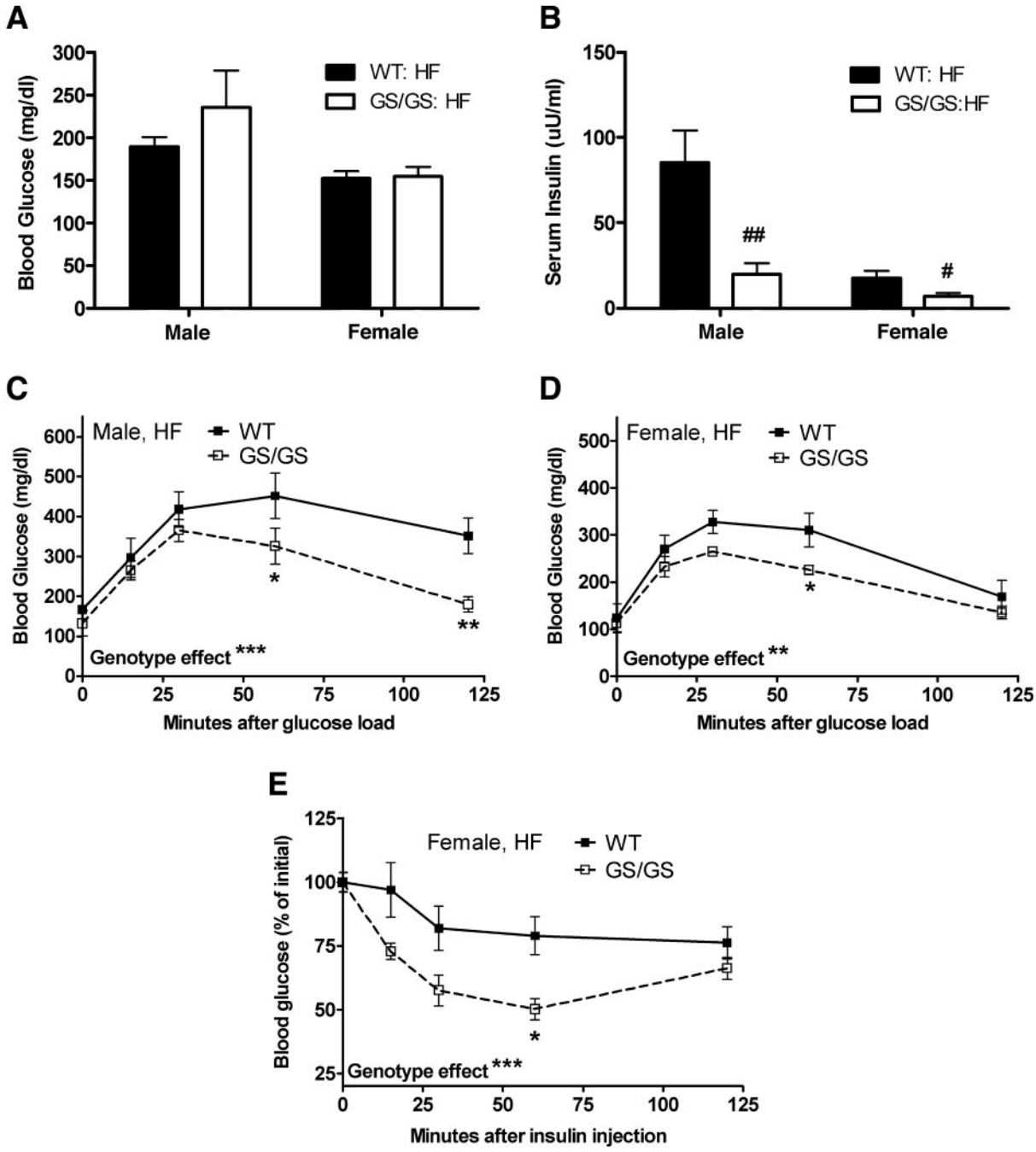

FIG. 5. Improved insulin sensitivity in $G\alpha_{i2}^{G184S}$ homozygote (GS/GS) mice. After a 25-week high-fat (HF) diet challenge, blood glucose (*A*) or plasma insulin concentration (*B*) in mice were measured after a 5-h fast, as described in RESEARCH DESIGN AND METHODS. Intraperitoneal glucose tolerance test in male (*C*) and female (*D*) mice was also measured. Mice were fasted overnight, and then glucose was injected intraperitoneally (2 mg/g body wt). *E*: Insulin tolerance tests were performed on 20-week-old female mice after a 5-week high-fat diet challenge. Mice were fasted for 4 h in the morning and injected intraperitoneally with regular insulin (0.5 unit/kg body wt). Changes in blood glucose were monitored over time. Genotype effect: $^{**}P < 0.001$, $^{***}P < 0.0001$ by two-way ANOVA. Significant differences at specific times: $^{*}P < 0.05$, $^{**}P < 0.01$ by two-way ANOVA with Bonferroni posttest; $\#P < 0.05$, $\#\#P < 0.01$ by *t* test. Data are means ± SE; $n = 3$–7 mice/group. WT, wild type.

why the decreased insulin levels in $G\alpha_{i2}^{G184S}$ mice do not result in hyperglycemia and/or impaired glucose tolerance.

$G\alpha_{i2}$ also plays a significant role in adipose tissue function. Many $G\alpha_i$-coupled receptors have strong antilipolytic actions mediated by pertussis toxin–sensitive mechanisms (32), including α_{2a}-adrenergic, A1 adenosine, and prostaglandin receptors and the recently deorphanized receptor for nicotinic acid (12). However, this might be expected to lead to enhanced accumulation of fat in our mice (33). In addition to its antilipolytic actions, $G\alpha_i$ signaling has been demonstrated to play a role in fat cell differentiation. $G\alpha_{i2}$ mRNA levels decrease during 3T3-L1 preadipocyte differentiation into adipocytes (34), and nearly all cocktails used to induce adipocyte differentiation in culture include the adenosine antagonist IBMX (3-isobutyl-1-methylxanthine), suggesting that Gi activation (perhaps by reducing cAMP) prevents differentiation. Activation of Gi-coupled LPA1 (lysophosphatidic acid 1) receptors or overexpression of A1 adenosine receptors reduced differentiation of preadipocytes (35,36), and LPA1

receptor–deficient mice became obese (35). Thus, reduced adipose differentiation could play a role in the reduced-fat phenotype.

Besides direct actions on fat tissue, there are other potential peripheral mechanisms for the decreased fat mass in the $G\alpha_{i2}^{G184S}$ mice. Hepatic G_s signaling pathways play a role in glucose and lipid regulation (37). Liver-specific $G\alpha_s$ knockout mice had increased liver weight and glycogen content but reduced adiposity and increased glucose uptake in liver and muscle. Thus, enhanced $G\alpha_{i2}$ signaling in liver with reduced cAMP levels could produce a similar phenotype; however, effects of $G\alpha_s$ disruption systemically are quite complex as a result of imprinting at the GNAS locus with either hypermetabolic or obesity phenotypes depending on maternal of paternal inheritance (38). In addition, increased sympathetic tone could lead to reduced adiposity. Male $G\alpha_{i2}^{G184S}$ mice exhibited increased heart rate and physical activity during telemetry electrocardiogram monitoring (26), consistent with a contribution of central sympathetic activation. Indeed, we see (Fig. S5) an increase in urinary norepinephrine excretion in male, but not female, G184S mice that correlates with their lower adiposity.

What could account for enhanced insulin responses in these mice? In the periphery, glucose uptake into skeletal muscle and adipocytes is mediated by translocation of GLUT4 from intracellular vesicles to the plasma membrane (39). A variety of GPCRs have been demonstrated to modify insulin-induced GLUT4 translocation. G_s-mediated signaling negatively regulates insulin-induced GLUT4 translocation. Heterozygous *GNAS*-deficient mice show an increased insulin sensitivity due to enhanced insulin-dependent glucose uptake into the skeletal muscle (40,41). Consistent with this, transgenic mice expressing a constitutively active mutant of $G\alpha_s$Q227L in fat, liver, and skeletal muscle showed decreased glucose tolerance (42). In contrast, G_i family G proteins seem to facilitate insulin actions. Pretreatment of isolated adipocytes and soleus muscle with pertussis toxin results in reduced insulin-stimulated glucose uptake (43). When $G\alpha_{i2}$ was downregulated in liver and adipose tissue using an antisense RNA approach, insulin resistance developed due to increased protein tyrosine phosphatase-1B activity (44). Additionally, mice expressing a constitutively active mutant of $G\alpha_{i2}$Q205L in fat, liver, and skeletal muscle displayed reduced fasting blood glucose levels and increased glucose tolerance (45). Adipocytes from these mice showed enhanced insulin-induced glucose uptake and GLUT4 translocation, as well as increased phosphatidylinositol 3-kinase and Akt activities (46). Interestingly, we previously showed that embryo fibroblasts from our mice had enhanced Akt activation by lysophosphatidic acid (30). Furthermore, ligand-dependent autophosphorylation of the human insulin receptor is positively regulated by $G\alpha_{i2}$ proteins (47). However, the level at which the insulin receptor–mediated pathway interacts with the G_i-mediated pathway remains unclear. It has even been suggested that G_i/G_o family G proteins physically interact with insulin receptor (48). Therefore, our results are consistent with this rather large, but not often appreciated, body of literature indicating important contributions of $G\alpha_{i2}$ in metabolic regulation. The RGS-insensitive $G\alpha_{i2}^{G184S}$ mice described here provide a more physiological model to investigate the mechanism of interaction between $G\alpha_{i2}$ protein signaling and insulin actions in vivo. Identifying the tissue site of $G\alpha_{i2}^{G184S}$ effects, such as in adipocytes, muscle, or brain, where hypothalamic insulin action via phosphatidylinositol 3-kinase may contribute to glucose homeostasis (49), will be an important future goal.

In conclusion, we have demonstrated that RGS-insensitive $G\alpha_{i2}^{G184S}$ mice exhibit resistance to the metabolic effects of a high-fat diet. Male $G\alpha_{i2}^{G184S}$ mice show a reduction in body weight and body fat mass, while males as well as females show improved glucose tolerance and insulin sensitivity. Future studies will aim to explore the molecular basis underlying this phenomenon. As a first step toward understanding the role of the RGS proteins and $G\alpha_{i2}$ signaling in body weight and glucose homeostasis, it will be important to identify the specific tissues and RGS proteins mediating these effects. This could open the door to a role for pharmacologic inhibition of RGS proteins as one element in control of obesity and impaired glucose tolerance associated with the chronic intake of fatty diets.

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