

Variation in Type 2 Diabetes–Related Traits in Mouse Strains Susceptible to Diet-Induced Obesity

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C57BL/6J (B6) and AKR/J (AKR) inbred strains of mice develop a comparable degree of obesity when fed a high-fat diet. However, although obese B6 mice are more glucose intolerant, obese AKR mice are more insulin resistant. To understand the basis for these strain differences, we characterized features of adiposity and glucose homeostasis in mice fed a high-fat diet for 8 weeks. The results indicated that despite hyperglycemia and impaired glucose tolerance, B6 mice have lower plasma insulin and are more insulin sensitive than AKR mice. Compared with adipose tissue of AKR mice, adipose tissue of B6 mice contained about threefold higher levels of total membrane-bound GLUT4 protein, whereas in skeletal muscle the levels were similar. Uptake of 2-[¹⁴C]deoxyglucose in vivo was reduced by a high-fat diet in adipose tissue, but not in skeletal muscle. Surprisingly, no significant differences in uptake occurred between the strains, despite the differences in GLUT4; however, glucose flux was calculated to be slightly higher in B6 mice. Higher expression of PEPCK in the liver of B6 mice, under both standard-diet and high-fat-diet conditions, suggests a plausible mechanism for elevated glycemia in these mice. In conclusion, phenotypic variation in insulin resistance and glucose production in the B6 and AKR strains could provide a genetic system for the identification of genes controlling glucose homeostasis. *Diabetes* 52:1958–1966, 2003

It is clear that obesity constitutes a risk factor contributing to the development of type 2 diabetes (1). However, there is also considerable evidence that individuals with similar levels of obesity can vary in their subsequent development of metabolic disorders like insulin resistance and diabetes (2). This suggests that there are environmental and genetic factors that increase the susceptibility of some individuals to developing diabetes. Numerous studies have shown the importance of adipose tissue for maintaining glucose homeostasis of the organism. In cases of extreme deficiency of adipose tissue, such as human lipodystrophies and transgenic mouse models of lipodystrophy, severe

diabetes is produced (3). However, lipodystrophic models do not really address the differences in the development of diabetes that arise in individuals with apparently similar levels of obesity. Instead, useful models to investigate this problem can be found among inbred strains of mice that are sensitive to diet-induced obesity.

Several mouse strains have been described in the literature, including those by Surwit et al. (4), West et al. (5), and Reifsnnyder et al. (6), in which susceptibility to obesity and diabetes has been found to be strain dependent. Attie and colleagues (7,8) have also identified quantitative trait loci (QTL) that predispose to the development of diabetes in the presence of severe obesity in mice homozygous for *Lep^{ob}*. These studies by Attie's group represent an extension of the work initially conducted by Coleman and Hummel (9), who found that the diabetes phenotype in mice homozygous for *Lep^{ob}* or *Lep^{R^{db}}* depended on whether the genetic background was B6 or C57BL/Ks. Surwit et al. (10) fed B6 and C57BL/Ks mice a high-fat/high-sucrose diet and found that C57BL/Ks mice were more resistant to diet-induced obesity compared with B6 mice and did not develop diabetes. The severe diabetes of *Lep^{ob/Lep^{ob}}* mice on a C57BL/Ks background found by Coleman must be attributable to the selective background effects interacting with leptin deficiency. In another model, genetic analysis of diabetes in NON and NZO mice has identified several QTLs controlling the trait; however, the physiological basis for the differences in the development of diabetes remains unknown (11,12).

B6 and AKR inbred strains are established models for diet-induced obesity (4,5,13). B6 mice will develop severe obesity, hyperglycemia, and insulin resistance if weaned onto a high-fat diet (4,14). Similar to B6 mice, AKR mice fed a high-fat diet substantially increase carcass lipid content but are less hyperglycemic (5). To understand the mechanism determining how individuals with seemingly similar levels of obesity vary in their susceptibility to diabetes, we analyzed the phenotypes of diet-induced obesity and diabetes in B6 and AKR mice. Our results indicated that despite comparable levels of obesity, B6 and AKR strains differ markedly in the regulation of glucose homeostasis and insulin sensitivity and that these strains might be useful in the identification of genes involved in the development of insulin resistance.

RESEARCH DESIGN AND METHODS

Animals. Male B6 and AKR mice age 4.5 weeks were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were maintained on a 12-h light/dark cycle at 24°C and fed a standard, low-fat laboratory diet ad libitum (PicoLab Rodent Diet 20 No. 5053; 12 kcal% fat, 64 kcal% carbohydrate, and 24 kcal% protein). At age 2 months, the animals ($n = 25$ in each group) were

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2-DG, 2-deoxyglucose; 2-DG-6-P, 2-DG 6-phosphate; QTL, quantitative trait loci; TG, triglyceride.

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TABLE 1
Body weight of mice at age 2 months (initial weight) and after 8 weeks on a standard or high-fat diet

Diet	<i>n</i>	Initial weight (g)	Week 8 weight (g)	Week 8 weight gain
Standard				
AKR/J	39	28.95 ± 2.26	32.69 ± 3.48	3.74 ± 1.86
B6	40	23.29 ± 1.22	27.03 ± 1.77	3.74 ± 1.19
<i>P</i>	—	<0.0001	<0.0001	0.99
High fat				
AKR/J	41	28.95 ± 2.49	45.47 ± 5.09	16.51 ± 3.39
B6	41	23.52 ± 1.43	38.30 ± 3.98	14.78 ± 3.09
<i>P</i>	—	<0.0001	<0.0001	0.018

Data are means ± SD of *n* animals in each group. *P* values were determined by single-factor ANOVA.

placed into single cages and fed the standard diet or a high-fat diet (Research Diets, New Brunswick, NJ) containing 58 kcal% fat, 26 kcal% carbohydrate, and 16 kcal% protein. Body weight was monitored every 2 weeks and food consumption was monitored weekly. After 8 weeks on the diet, mice were first given an insulin tolerance test or glucose tolerance test and then killed by cervical dislocation. White adipose tissue from femoral (subcutaneous) and epididymal fat depots, liver, and pancreas and samples of skeletal muscle (*quadriceps femoris*) were dissected, frozen in liquid nitrogen, and stored at -75°C for further analysis.

Insulin and glucose tolerance tests. Insulin sensitivity was tested by an insulin tolerance test given to fed mice injected intraperitoneally with regular human insulin (0.75 units/kg body wt; Lilly, Indianapolis, IN) at 1:00 P.M. Tail blood was sampled at 0 (baseline), 15, and 30 min after the injection. Blood glucose was measured by the use of One Touch II Meter (LifeScan, Milpitas, CA), and insulin sensitivity was assessed by calculating the slope of the time-dependent decrease in glucose concentration after the injection of insulin (15,16). A glucose tolerance test was performed in a separate group of mice that were fasted overnight (~16 h) and then at 10:00 A.M. injected intraperitoneally with D-glucose (1 g/kg body wt). Blood was collected at 0 (fasting levels), 15, 30, 60, and 120 min after glucose injection, and the blood glucose concentration was immediately measured as above.

Blood glucose and plasma parameters. Truncal blood was obtained after mice were killed and collected into EDTA-containing tubes. Samples were kept on ice, then centrifuged at 5,000g for 10 min at 4°C. Plasma was analyzed for insulin, triglyceride (TG), and free fatty acid levels. Insulin was measured with a sensitive rat insulin radioimmunoassay kit (Linco Research, St. Charles, MO), and TGs and free fatty acids were measured by enzymatic colorimetric assays from Sigma Diagnostics (Procedure No. 320-UV) and Wako Chemicals (Richmond, VA; procedure NEFA C), respectively.

DNA measurements. Tissue cellularity was examined by fluorometric quantitation of DNA in samples of epididymal and femoral fat according to the method of Labarca and Paigen (17) using a fluorescent dye Hoechst No. 33258 (Sigma).

Counter counter. Adipose tissue cellularity was investigated using OsO₄ fixation, followed by electronic counting and sizing (18,19). Briefly, 50 mg of epididymal adipose tissue were placed into a scintillation vial containing 4 ml of OsO₄ solution and 2.4 ml collidine-HCl buffer. After 1 week at room temperature, fixed tissue was washed onto a 20-μm nylon screen with 0.154 mol/l NaCl (saline). The sample was then kept in a vial with 10 ml saline for

~24 h, then washed again onto the 20-μm nylon screen with saline and incubated in 10 ml 8 mol/l urea. After several weeks, fixed adipocytes were separated from tissue debris by filtering the samples through a 250-μm nylon screen and collecting the adipocytes onto the 20-μm screen. The cells were rinsed into beakers containing 300 ml saline; 3 ml 0.1% Triton X-100 were added to prevent cell aggregation. Adipocyte number and size distribution were measured electronically in aliquots of continuously stirred cell suspension using Multisizer 3 (Beckman Coulter) equipped with a 400-μm aperture. **Gene expression analysis.** Total RNA was extracted from liver and adipose tissue samples (50 and 100 mg, respectively) using Tri Reagent (Molecular Research Center, Cincinnati, OH), after which 20 μg of total RNA were loaded onto a 1.2% agarose-formaldehyde gel. Gene expression in the liver was analyzed by Northern blot according to the method of Derman et al. (20) using a full-length PEPCK cDNA probe. Gene expression in adipose tissue was analyzed by quantitative real-time RT-PCR using the ABI Prism 7700 sequence detection system (Applied Biosystems). PCR primers and TaqMan probes were designed using GenBank sequences NM 013462 and AB008453 for β3-adrenergic receptor and GLUT4, respectively.

Tissue triglycerides. Tissue fragments were dissolved in alcoholic KOH according to the method of Salmon and Flatt (21). Deliberated glycerol was assayed by a coupled enzyme system from Sigma Diagnostics (Procedure No. 320-UV). The tissue TG concentration was calculated using an average molecular weight of 885/molecule of TG.

Immunoblotting. Membrane fractions were isolated according to the method of Ezaki et al. (22). In our modified protocol, homogenates were precentrifuged at 600g for 2 min to remove tissue debris. The protein content of solubilized membranes was assessed by the improved Lowry assay (Bio-Rad, Hercules, CA). Membrane samples (40 μg) were incubated for 10 min at 37°C in a solution containing (f.c.): 10% (vol/vol) glycerol, 62.5 mmol/l Tris (pH 6.8), 2.5% (wt/vol) SDS, 0.5% (wt/vol) bromophenol blue, 5% (vol/vol) 2-mercaptoethanol, and 8 mol/l urea. SDS-PAGE was performed according to the method of Laemmli (23) using a 5% stacking gel and 10% resolving gel. After an electrophoretic transfer of proteins onto Immobilon-P membranes (Millipore, Bedford, MA), GLUT4 was detected using a 1:5,000 dilution of rabbit anti-GLUT4 primary antibody (Cat. No. AB1346; Chemicon International, Temecula, CA), a 1:20,000 dilution of donkey anti-rabbit IgG coupled with horseradish peroxidase (Cat. No. NA 9340; Amersham Pharmacia Biotech, Piscataway, NJ), and an enhanced chemiluminescence kit from Amersham (Cat. No. RPN 2109). The signal on the film was quantified by densitometry.

TABLE 2
Blood glucose levels and plasma concentrations of insulin, total triglycerides, and free fatty acids

Diet	Blood glucose (mg/dl)	Insulin (ng/ml)	Triglyceride (mg/dl)	Free fatty acid (mmol/l)
<i>n</i>	24	17–19	11–12	17–19
Standard				
B6	117 ± 3	0.73 ± 0.12	87 ± 3	0.63 ± 0.03
AKR	96 ± 2	1.12 ± 0.16	141 ± 7	0.60 ± 0.03
<i>P</i>	<0.0001	0.0666	< 0.0001	0.4797
High fat				
B6	132 ± 4	1.87 ± 0.28	143 ± 6	0.75 ± 0.03
AKR	100 ± 2	4.60 ± 0.51	171 ± 9	0.61 ± 0.03
<i>P</i>	<0.0001	< 0.0001	0.0129	0.0011

Data are means ± SE of *n* animals in each group. Insulin, triglycerides, and free fatty acids were analyzed in plasma samples obtained from the blood of overnight-fasted (10–12 h) animals after they were killed. Blood glucose levels were determined in mice in the middle of the inactive period (~1:00 P.M.). *P* values were determined by single-factor ANOVA.

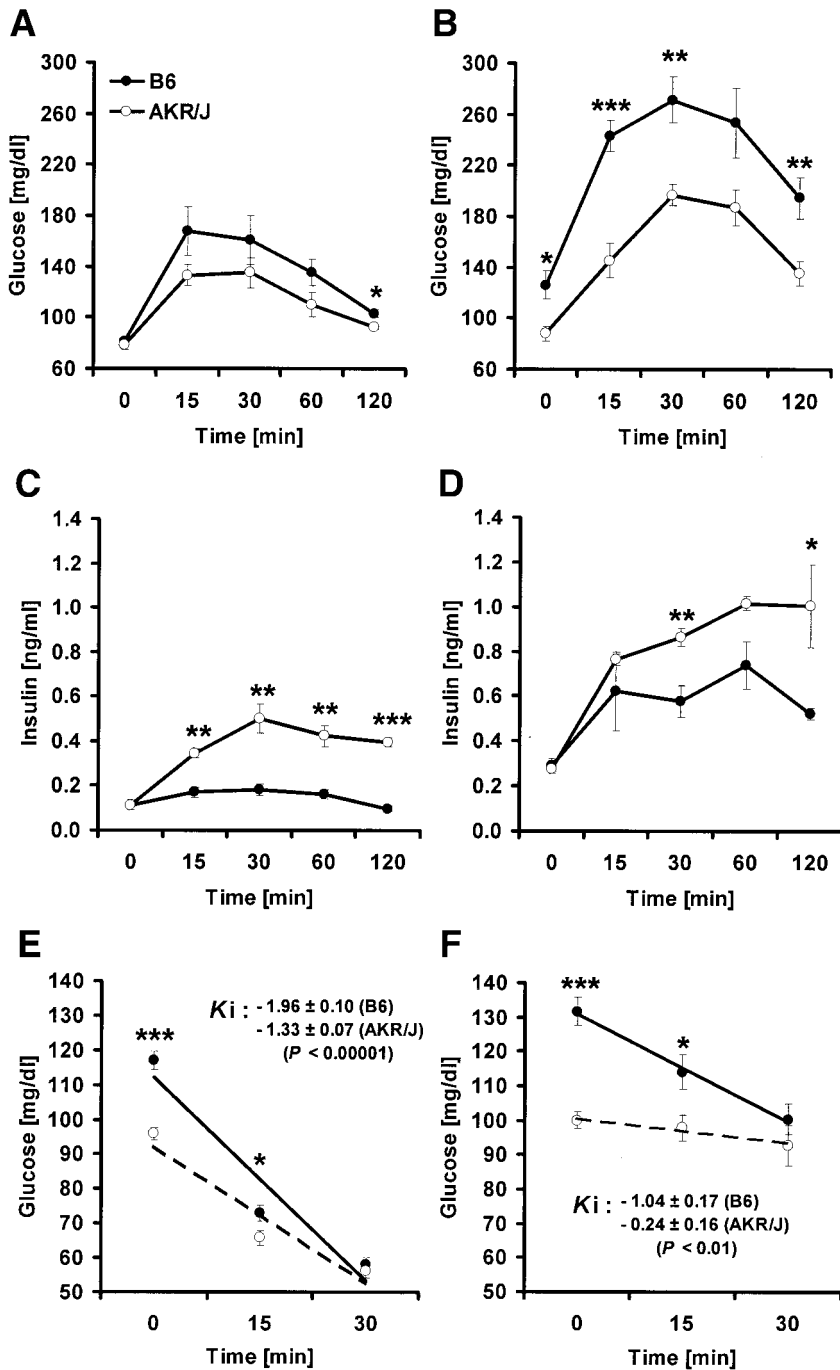


FIG. 1. *A* and *B*: Glucose tolerance test in AKR and B6 mice fed a standard (*A*) or high-fat diet (*B*). After an overnight fast (~16 h), B6 and AKR mice were injected intraperitoneally with glucose (1 g/kg body wt). Blood glucose was assessed before ($t = 0$) and 15, 30, 60, and 120 min after the injection of glucose. Every time point is a mean \pm SE ($n = 6$). *C* and *D*: Insulin secretion from pancreas during glucose tolerance test in mice fed standard (*C*) and high-fat diet (*D*). Data are means \pm SE ($n = 2$, where each sample represents pooled plasma from at least three mice because of low sample volumes). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ for B6 vs. AKR fed the same diet. *E* and *F*: Insulin tolerance test in mice fed a standard (*E*) or high-fat diet (*F*) for 8 weeks. Mice were injected intraperitoneally with regular human insulin (0.75 units/kg body wt) at 1:00 P.M. and blood was sampled at 15 and 30 min after the injection. Insulin sensitivity (K_i) was measured as the slope of the fall in blood glucose. Results are means \pm SE of 24–25 mice for every time point. * $P < 0.05$; *** $P < 0.001$ for B6 vs. AKR fed the same diet. ●, B6 mice; ○, AKR mice.

The specificity of the primary antibody was verified by including a blocking peptide (Cat. No. AG677; Chemicon International).

In vivo glucose uptake. The effect of the high-fat diet on in vivo glucose uptake in tissues of B6 and AKR mice was determined by injecting 10 μ Ci of 2-deoxy-D-[1- 14 C]glucose intraperitoneally and insulin (0.75 units/kg body wt) into mice fasted overnight (~12 h). After 1 h, tissues were removed and frozen in liquid nitrogen. Radiolabeled 2-deoxyglucose (2-DG) and the phosphorylated form were extracted and separated from each other by ion exchange chromatography, as described (24). Relative uptake was calculated as the ratio of 2-[14 C]deoxyglucose to its phosphorylated form.

Statistics. Data are given as means \pm SE. Comparisons between datasets were analyzed by single-factor ANOVA (Microsoft Excel). $P < 0.05$ was considered significant.

RESULTS

Induction of obesity in AKR and B6 mice. At age 2 months, male AKR and B6 mice, which differed in their

initial body weights by ~5 g, each gained 3.74 g after being fed the low-fat diet for 8 weeks (Table 1). In a second cohort of mice fed a high-fat diet (58 kcal% fat), AKR mice gained 16.51 g, a 57% increase over the initial body weight, whereas B6 mice gained 14.78 g, a 63% increase. Although the absolute increase in weight gain reached statistical significance, these two strains are very similar in their susceptibility to obesity when fed a high-fat diet. Cumulative food intake in kilocalories in a subgroup of 10 mice described in Table 1 was ~15% higher in AKR mice. Intake of the standard diet for AKR and B6 was 821 ± 16 and 704 ± 9 kcal, respectively, and for the high-fat diet, was $1,002 \pm 23$ and 820 ± 19 kcal, respectively. Body weight gained in milligrams per kilocalorie of food consumed was

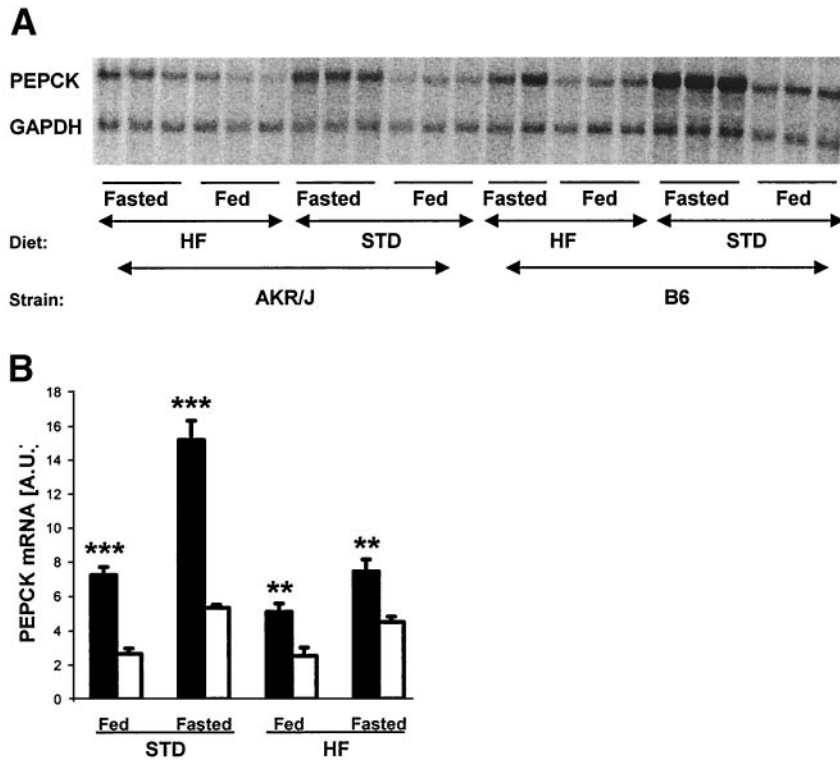


FIG. 2. effect of a high-fat diet on PEPCK expression in the liver. **A:** Representative Northern blot analysis of PEPCK gene expression in B6 and AKR mice fed a standard (STD) or high-fat (HF) diet. Equal amounts (20 μ g) of total RNA were loaded. Animals were fasted overnight (\sim 12 h). **B:** Quantification of the results from (A). Bars are means \pm SE ($n = 6$). ■, B6; □, AKR; ** $P < 0.01$; *** $P < 0.001$ for B6 vs. AKR fed the same diet.

increased \sim 3.5-fold in both strains by the high-fat diet (AKR, 17.8 ± 0.4 and B6, 19.5 ± 0.7 ; $P < 0.05$). The data suggest that AKR mice eat more because of a larger lean body mass. The increase in adiposity in these mice was confirmed by analysis of individual fat depots. The relative distribution of fat among the major individual fat depots (epididymal, retroperitoneal, and femoral fat) was similar between AKR and B6 mice.

Glucose tolerance and insulin sensitivity were unlinked in mice with diet-induced obesity. A modest, but highly significant, hyperglycemia developed in B6 mice compared with AKR mice fed the high-fat diet for 8 weeks (Table 2). The development of diet-induced hyperglycemia in B6 mice was previously described by Surwit et al. (4). In contrast, a more robust increase in plasma insulin levels occurred in AKR mice than in B6 mice fed the high-fat diet, despite higher blood glucose in B6 mice. Plasma TGs were marginally elevated by the high-fat diet, particularly in AKR mice, and free fatty acids were higher in B6 mice (Table 2).

B6 and AKR mice fed the low-fat diet showed a slight difference in glucose tolerance (Fig. 1A). When fed the high-fat diet, both strains showed increased glucose intolerance; however, the glucose intolerance was much more severe in B6 mice (Fig. 1B). One would have predicted that increased insulin resistance would have accompanied higher glucose intolerance in B6 mice and that plasma insulin levels during the glucose tolerance test would have been higher in B6 mice. In contrast, plasma insulin levels during the glucose tolerance test were already more robust in the AKR mice fed the standard diet (Fig. 1C) and although the high-fat diet increased insulin levels in both strains (15 min postinjection), the insulin levels in B6 mice were sustained at much lower levels than in AKR mice, suggesting the failure of a second-phase insulin response

(Fig. 1D). Consistent with these indications of differences in the control of plasma insulin levels, the insulin tolerance tests showed that AKR mice fed a standard diet were less responsive to insulin than B6 mice and that insulin resistance in mice fed the high-fat diet was much more severe in the AKR strain (Fig. 1E and F). Estimates of insulin sensitivity (see RESEARCH DESIGN AND METHODS) indicated that on the low-fat diet, B6 mice were 50% more sensitive than AKR mice (Fig. 1E); however, on the high-fat diet, the insulin sensitivity was reduced 2-fold in B6 mice versus 5.5-fold in AKR mice, making the AKR mice 4-fold less sensitive than B6 mice (Fig. 1F). Consistent with the marked insulin resistance in AKR mice, the short-term high-fat diet feeding led to a compensatory hyperinsulinemia in this strain, whereas the relatively low insulin levels in hyperglycemic B6 mice indicated that these mice did not completely compensate (Table 2). The divergent patterns of insulin sensitivity and glucose tolerance in B6 and AKR mice suggested independent, parallel effects of the high-fat diet at different levels of glucose homeostatic system.

Increased gluconeogenesis in the liver of B6 mice. The expression of PEPCK, a key enzyme involved in gluconeogenesis, was much higher in B6 mice than in AKR mice fed the standard diet (Fig. 2A and B). Although the difference between strains diminished on the high-fat diet, PEPCK expression was still significantly higher in B6 mice. As expected, fasting significantly elevated liver PEPCK mRNA in standard diet-fed mice of both strains. Interestingly, the expression of PEPCK in response to fasting was diminished in the B6 mice fed the high-fat diet, whereas in AKR mice it was similar to their standard diet-fed counterparts. These data indicate increased gluconeogenesis in the liver of B6 compared with AKR mice. Because there was no strain difference in glucose uptake

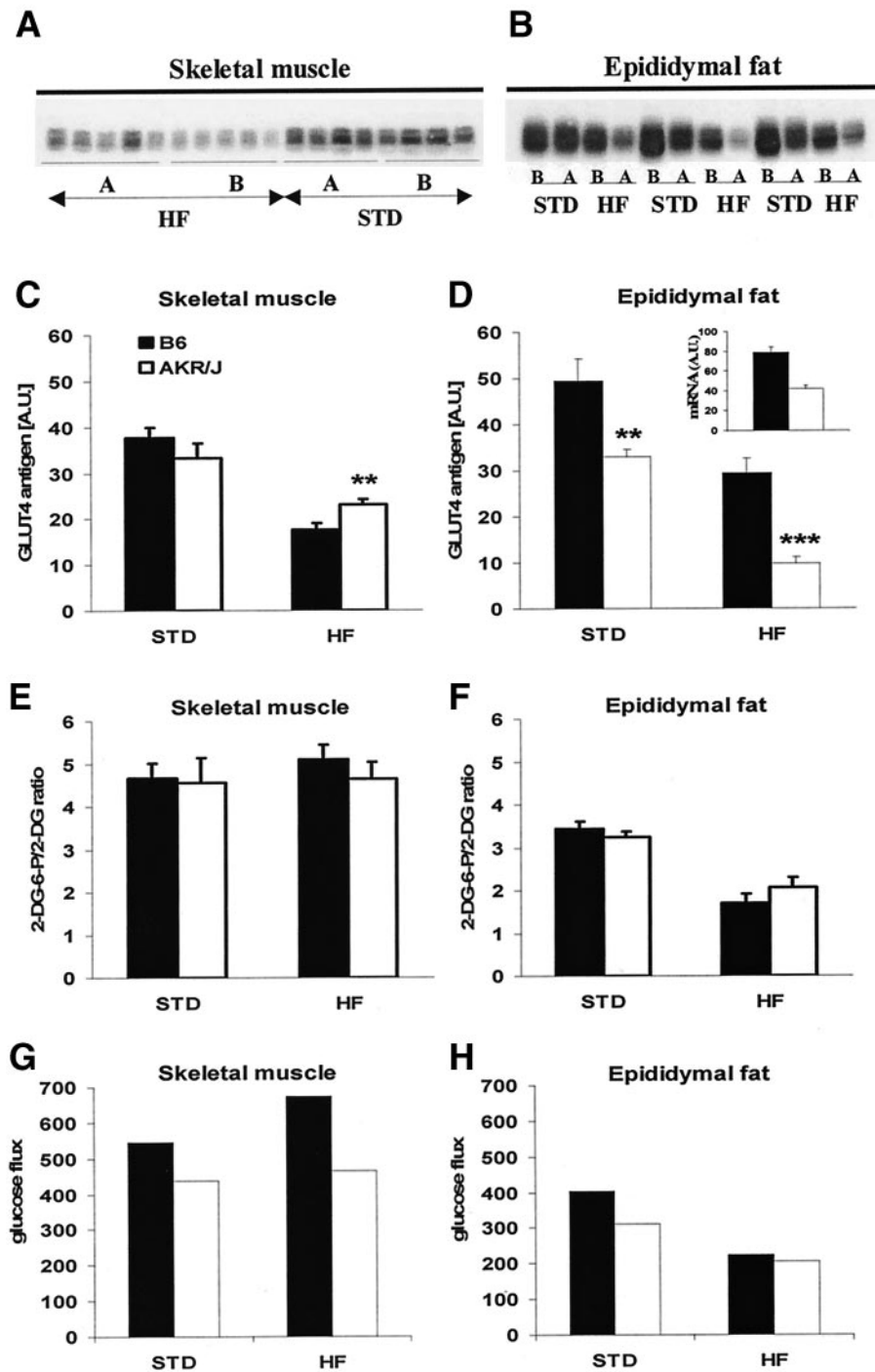


FIG. 3. A–D: Effect of a high-fat diet on total membrane-bound GLUT4 in insulin-responsive tissues. A and B: Representative Western blot analyses of GLUT4 in a total membrane fraction isolated from *quadriceps femoris* muscle (A) and epididymal adipose tissue (B) of B6 (B) and AKR (A) mice fed a standard (STD) or high-fat (HF) diet. C and D: Quantification of the data from A and B. Bars represent means \pm SE of 12–19 mice (skeletal muscle; C) and 5–11 mice (epididymal fat; D). Inset: GLUT4 mRNA in epididymal fat of mice fed HF diet ($n = 17$ –18; $P < 0.001$). ** $P < 0.01$; *** $P < 0.001$ for B6 (■) vs. AKR (□) fed the same diet. E–H: Glucose uptake into insulin-responsive tissues of B6 and AKR mice in vivo. Mice were fed either STD or HF diet for 8 weeks and then injected intraperitoneally with 2-deoxy-D-[1- 14 C]glucose (10 μ Ci, bolus) and insulin (0.75 units/kg body wt) after an overnight fast (~ 12 h). After 1 h, tissues were removed and both [14 C]2-DG-6-P and [14 C]2-DG in epididymal fat (E) and skeletal muscle (F) were quantified and expressed as a ratio. No significant differences between strains were detected on either diet. Estimates of in vivo glucose flux into tissues (G and H) were made by multiplying the 2-DG 6-P to 2-DG ratio by the blood glucose levels shown in Table 3.

in liver in vivo (see below), the effect on PEPCK may have been caused by differences in circulating insulin levels rather than insulin sensitivity.

Lower amounts of GLUT4 in peripheral tissues after high-fat feeding. It has been well documented that reductions in GLUT4 protein levels are associated with increased insulin resistance (25). Accordingly, changes in GLUT4 protein levels in total membrane preparations occurred in both skeletal muscle and adipose tissue of mice fed the high-fat diet (Fig. 3A and B). In skeletal muscle (Fig. 3A and C), the levels of GLUT4 were depressed in B6 and AKR mice fed the high-fat diet, but slightly more so in B6 than in AKR mice. Also, in epidid-

ymal adipose tissue, the content of membrane-bound GLUT4 was significantly decreased by the high-fat diet in both mouse strains; however, the reductions in AKR mice were much greater than those in B6 mice (Fig. 3B and D). Although GLUT4 decreased $\sim 40\%$ in the B6 mice, the GLUT4 content dropped $\sim 70\%$ in the AKR mice from the levels found in the standard diet-fed counterparts. GLUT4 mRNA was about two-fold higher in the epididymal fat depot of B6 mice (79 ± 5 [B6] vs. 42 ± 3 a.u. [AKR]), suggesting that the strain differences in GLUT4 antigen levels may be determined in part at the level of mRNA production (Fig. 3D, inset). Thus, the higher GLUT4 content in epididymal fat of B6 mice fed the standard or

TABLE 3
Tissue weight, DNA content, and weight/DNA ratios of epididymal and femoral fat pads

Diet	Epididymal fat			Femoral fat		
	Weight (mg)	DNA (μg)	Weight/DNA (mg/ μg)	Weight (mg)	DNA (μg)	Weight/DNA (mg/ μg)
<i>n</i>	17–19	15–19	15–19	17–19	14–16	14–16
Standard						
B6	445 \pm 40	179 \pm 8	2.67 \pm 0.16	224 \pm 17	156 \pm 20	1.66 \pm 0.18
AKR	1,006 \pm 83	193 \pm 17	5.61 \pm 0.57	445 \pm 32	260 \pm 38	1.95 \pm 0.19
<i>P</i>	<0.0001	0.4864	0.0001	<0.0001	0.0213	0.2908
High Fat						
B6	2,070 \pm 148	433 \pm 46	5.05 \pm 0.27	841 \pm 56	268 \pm 18	3.23 \pm 0.14
AKR	2,916 \pm 80	737 \pm 65	4.44 \pm 0.34	1,436 \pm 117	414 \pm 32	3.50 \pm 0.10
<i>P</i>	<0.0001	0.0005	0.1703	<0.0001	0.0006	0.1355

Data are means \pm SE of *n* animals in each group. Cellularity of epididymal and femoral fat pads was estimated by fluorometric quantitation of DNA.

high-fat diet might contribute to the increased insulin sensitivity of B6 mice compared with AKR mice through the increased capacity for GLUT4-mediated glucose uptake into adipose tissue.

In vivo glucose uptake reduced in adipose tissue but not in skeletal muscle of high-fat-fed mice. Previously available data on GLUT4 protein levels suggested that insulin-stimulated glucose uptake into adipose tissue would be reduced in mice fed the high-fat diet and that uptake of glucose into adipose tissue would be lower in AKR than B6 mice. However, in our study, the insulin-stimulated uptake of 2-DG into epididymal fat performed in vivo confirmed only the first assumption. In the skeletal muscle of both strains, 2-DG uptake was unchanged after 8 weeks on the high-fat diet (Fig. 3E). Glucose transport into adipose tissue was reduced about two-fold in B6 and AKR mice fed the high-fat diet; however, contrary to expectations based on GLUT4 levels, there was no difference between strains (Fig. 3F). In addition to the analysis of glucose uptake in epididymal fat and skeletal muscle, a similar analysis was done on the femoral fat. Slight reductions occurred in the femoral fat of mice fed the high-fat diet (no differences between strains). An estimate of glucose flux into tissues was calculated from the ratios of 2-deoxyglucose 6-phosphate (2-DG-6-P) to 2-DG, and the blood glucose levels (data in Table 2) suggested that higher rates of glucose flux were occurring in tissues of B6 mice.

Adipocyte cell number and size and accumulation of TGs in nonadipose tissues of AKR compared with B6 mice. It was recently hypothesized that a higher capacity to store excess calories in adipose tissue prevents the development of insulin resistance and diabetes (26). Our results showed that AKR mice had larger fat depots compared with B6 mice when fed either a low- or high-fat diet (Table 3). Adipose tissue cell number, assessed by DNA quantification, tended to be higher in the AKR strain under both low- and high-fat conditions (Table 3). The high-fat diet caused a two- to fourfold increase in the total DNA content of both epididymal and femoral tissues, and resulted in AKR mice having about twice as much DNA in fat depots as B6 mice. Calculations of cell size (ratio of depot weight/DNA) indicated that only in the epididymal fat of standard diet-fed animals was the cell size larger in AKR mice, whereas in the femoral fat no significant

differences in cell size were found under either dietary condition (Table 3). The increase in cell size in epididymal fat of AKR mice fed a standard diet was also evident from the distribution of cell sizes estimated by Coulter counter analysis of osmium-fixed adipocytes (Fig. 4). No differences were detected in fat depots from mice fed the high-fat diet.

The accumulation of TGs was evaluated in nonadipose tissues, as it could be associated with lipotoxicity and insulin resistance (rev. in 27). In general, mice being fed the high-fat diet for 8 weeks resulted in a two- to fivefold increase in TG levels of nonadipose tissues (Fig. 5). In pancreas, similar to skeletal muscle, B6 mice fed the low-fat standard diet had lower tissue TG levels than AKR mice (29 \pm 6 vs. 77 \pm 22 mg/g; *P* < 0.05), but no significant difference was found between the strains fed the high-fat diet (144 \pm 31 [B6] vs. 166 \pm 36 mg/g [AKR]). In the liver of B6 mice, tissue TG concentrations increased from 38 \pm 5 mg/g in mice fed the low-fat standard diet to 102 \pm 7 mg/g in mice fed the high-fat diet. Correspondingly, in AKR mice, the TG levels were 22 \pm 2 mg/g on the standard diet and increased to 71 \pm 6 mg/g on the high-fat diet. In the skeletal muscle, standard diet-fed B6 mice had significantly lower tissue TG levels than AKR mice (24 \pm 5 [B6] vs. 40 \pm 5 mg/g [AKR]; *P* < 0.05); however, the high-fat diet increased muscle TG concentrations to a similar extent in both strains (100 \pm 20 [B6] vs. 94 \pm 15 mg/g [AKR]). Thus, insulin resistance in the AKR mice fed the high-fat diet could not be explained by the lower capacity of their fat depots to store TGs or by the excessive accumulation of TGs in nonadipose tissues.

DISCUSSION

To determine the mechanisms by which individuals with seemingly similar levels of obesity vary in their susceptibility to diabetes, we looked for a genetic model in which two strains of mice were susceptible to obesity when fed a high-fat diet, but which varied in their progression to diabetes. Previously, the B6 and A/J strains have been investigated as a genetic model of two strains of mice that differed in their sensitivity to diet-induced obesity. B6 mice are more sensitive to the development of obesity by a high-fat diet and, correspondingly, to increased hyperglycemia and insulin resistance (4), whereas A/J mice, being

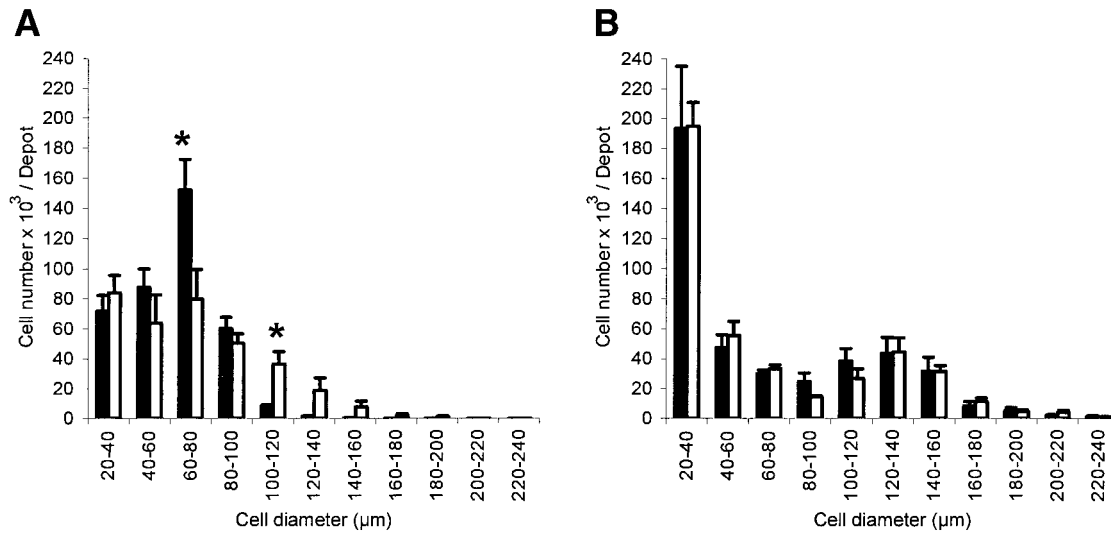


FIG. 4. Cellularity of epididymal adipose tissue. Coulter counter measurements of adipocyte size and number in fragments of epididymal adipose tissue from B6 (■) and AKR (□) mice fed either a standard (A) or high-fat diet (B). The mean adipocyte size in mice fed the standard diet was 63 ± 1 and 70 ± 3 μm ($P = 0.05$) in the B6 and AKR strain, respectively, whereas adipocyte size in animals fed the high-fat diet was 71 ± 2 and 70 ± 2 μm in B6 and AKR, respectively. Bars are means \pm SE ($n = 4$). * $P < 0.05$ for B6 vs. AKR fed the same diet.

resistant to diet-induced obesity, are normoglycemic and insulin sensitive. A limited genetic analysis of backcross progeny and AXB recombinant inbred from B6 and A/J suggests that hyperglycemia and insulin sensitivity are controlled by different genetic factors (15); however, no follow-up work has mapped specific QTLs determining the obesity and diabetes phenotypes. Recent investigations on the development of insulin resistance in B6 mice fed a high-fat diet have concluded that glucose intolerance is explained by reduced glucose effectiveness in early periods and insufficient early insulin secretion after long-term feeding (28). The mechanisms for these defects in B6 mice made obese from high-fat feeding is unknown.

We have now compared the consequences of diet-induced obesity in B6 and AKR mice on sub-phenotypes of adiposity and glucose homeostasis (Table 4). The most striking finding to emerge from this study was the large difference in the development of hyperglycemia and insulin resistance, even though the two strains of mice are equally sensitive to high-fat-diet-induced obesity. Similar to findings from previous studies, B6 mice on a high-fat diet were hyperglycemic and glucose intolerant and had low levels of

plasma insulin. This observation was consistent with the finding from a previous study that reduced insulin secretion contributes to glucose intolerance in B6 mice (28). Although the obese AKR mice were almost normoglycemic, they were much more insulin resistant than B6 mice, as evidenced by insulin intolerance tests. Elevated plasma insulin levels in AKR mice suggested that normoglycemia was maintained by a compensatory increase in insulin output. Although these strain differences in hyperglycemia and insulin resistance were more pronounced in mice fed a high-fat diet, they were already evident in mice on the standard diet for an equivalent period of time. These phenotypic differences in insulin resistance and glucose intolerance in B6 and AKR mice were robust, having been consistently observed in several groups of male mice over a 2-year period. These features of early insulin resistance, increased plasma insulin, and normoglycemia evident in the AKR mice resemble the classic descriptions for the prediabetic phase of type 2 diabetes in humans (29,30).

In addition to reduced insulin output by the pancreas, the PEPCK expression in liver suggests that B6 mice have increased hepatic glucose output. Excessive hepatic glu-

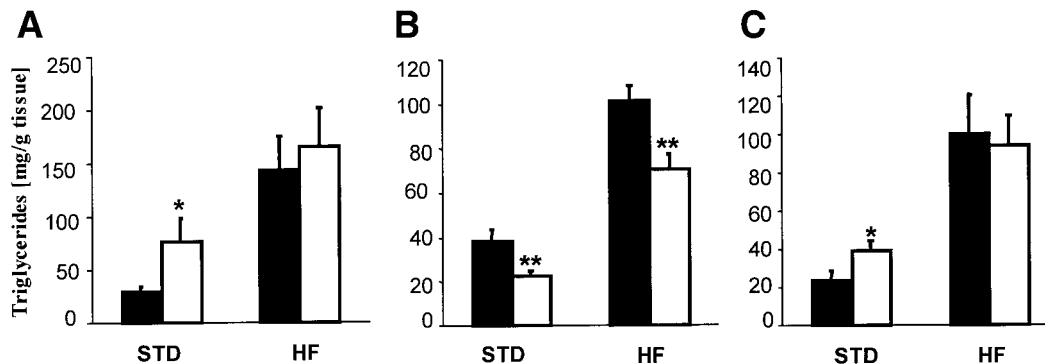


FIG. 5. Triglycerides in nonadipose tissues. TG concentration was assessed in pancreas (A), liver (B), and skeletal muscle (*quadriceps femoris*; C) of B6 (■) and AKR (□) mice fed a standard (STD) or high-fat (HF) diet. Tissue TGs were analyzed by saponification of neutral fats and subsequent enzymatic measurements of deliberated glycerol. Bars are means \pm SE ($n = 17-19$). * $P < 0.05$; ** $P < 0.01$ for B6 vs. AKR/J fed the same diet.

TABLE 4
Comparative effects of a high-fat diet on the subphenotypes of obesity and diabetes in AKR/J and B6 mice

Subphenotypes	AKR	B6
Adiposity	+++	+++
Hyperglycemia/glucose intolerance	+	+++
Plasma insulin	+++	+
Insulin resistance	++++	+
TG in non-adipocytes	+++	+++
Liver fasted PEPCK	+	++
GLUT4/adipose	---	-
Glucose uptake/adipose	-	-
GLUT4/muscle	--	--
Glucose uptake/muscle	N	+
Adipose tissue cellularity	+++	++
Adipose cell size	+	++

Shown is the expression of various phenotypes in mice fed a high-fat diet for 8 weeks compared with their counterparts fed the low-fat, standard diet. Relative changes are indicated by a number of pluses (+) or minuses (-). TG, triglycerides. N, no change.

cose production is a major contributor to both fasting hyperglycemia and exaggerated postprandial hyperglycemia in the type 2 diabetes (31). Because B6 mice are actually more insulin sensitive, it is likely that the reduced insulin levels rather than differences in insulin sensitivity would promote increased glucose output. We also evaluated glucose uptake by the muscle, epididymal, and inguinal fat depots to evaluate insulin resistance in AKR and B6 mice fed a low- and high-fat diet. Although the data obtained from the relative accumulation of ¹⁴C-labeled 2-DG showed a reduction in glucose uptake in adipose tissue in mice fed a high-fat diet, there were no differences in uptake between strains. Although these estimations of glucose uptake from the ratio of 2-DG 6-P to 2-DG (Fig. 3E and F) do not correlate to the marked changes in the levels of GLUT4 in epididymal fat and gastrocnemius skeletal muscle, calculations of glucose flux from these ratios and the differences in glucose levels of these mice suggest higher uptake of glucose in B6 mice (Fig. 3G and H). Accordingly, the higher GLUT4 levels in B6 mice may reflect an attempt to increase glucose flux into peripheral tissues in response to elevated blood glucose levels. Such a physiological response in B6 mice may be augmented by their enhanced insulin sensitivity. The role of GLUT4 downregulation in the pathogenesis of insulin resistance and glucose intolerance has been confirmed in mice with either adipose-selective (32) or muscle-selective (33) ablation of GLUT4. Interestingly, in human patients with obesity or type 2 diabetes, GLUT4 levels are not reduced in muscle, but are in adipose tissue (34,35). Our results suggest that a high-fat diet in mice is associated with a downregulation of GLUT4 in both adipose tissue and skeletal muscle; however, the degree of this downregulation is modified by the status of insulin sensitivity and hyperglycemia.

The accumulation of TGs in the nonadipose tissues, such as liver, muscle, and pancreas, has been proposed as an important mechanism causing insulin resistance (the lipotoxicity hypothesis) (27,30). Although we found a significant increase in TG levels in nonadipose tissue of both strains fed the high-fat diet, no differences in accumulation were evident that could account for the greater

insulin resistance in AKR mice (Fig. 4). Danforth (26) recently hypothesized that individuals with a larger capacity to store fat in adipose tissues could achieve protection against the accumulation of TGs in nonadipose tissues. In support of this hypothesis, it has been cited that 1) Zucker fatty rats that have improved glucose tolerance and insulin sensitivity after treatment with thiazolidinediones have also increased number of small adipocytes and larger fat depots (36–38); 2) animals with severe lipodystrophies are diabetic (39–41); and 3) enlarged adipocyte size is an independent predictor of diabetes in obese Pima Indians (42). We evaluated the characteristics of cell number by determining DNA content in tissue and cell size by Coulter counting of osmium-fixed adipocytes. In our study, the morphological data indicated that AKR mice, which are more insulin resistant, had larger fat depots (Table 3). DNA content suggests that both epididymal and femoral fat from AKR mice have an increased cell number (Table 3). Although the increase in DNA could be attributable to increases in stromal cells and endothelial cells, nevertheless, it is impressive how large an increase in DNA content occurred as a result of a high-fat diet: 2.4- to 3.8-fold in epididymal fat (Table 3). The data with osmium fixed cells showed an increase in larger adipocytes in both B6 and AKR mice fed a high-fat diet, but no differences between the strains (Fig. 5). On a standard diet, the AKR mice tended to have larger adipocytes. Our data indicated that both strains of mice responded to the increased lipid load caused by the high-fat diet by stimulating cell proliferation in the fat depots. Furthermore, the similarity in the response does not support the idea that differences in insulin sensitivity between the strains are determined by differences in adipocyte number. Nevertheless, this increase in cell number (DNA content) was not sufficient to prevent TG accumulation in liver, muscle, and pancreas.

In summary, we demonstrated that short-term high-fat feeding in B6 and AKR mice affects insulin sensitivity and glucose tolerance rather independently. Our results demonstrated that glucose intolerance can occur despite relatively good insulin sensitivity of peripheral tissues. The combination of relative insulin sensitivity and glucose intolerance in B6 mice compared with a marked insulin resistance and relative glucose tolerance in AKR mice during the initial periods of high-fat feeding can provide a useful model for studying basic mechanisms of glucose homeostasis and insulin resistance.

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