Opposite Effects of Background Genotype on Muscle and Liver Insulin Sensitivity of Lipoatrophic Mice

ROLE OF TRIGLYCERIDE CLEARANCE*

The metabolic phenotype of the A-ZIP/F-1 (AZIP) lipoatrophic mouse is different depending on its genetic background. On both the FVB/N (FVB) and C57BL/6J (B6) backgrounds, AZIP mice have a similarly severe lack of white adipose tissue and comparably increased insulin levels and triglyceride secretion rates. However, on the B6 background, the AZIP mice have less hyperglycemia, lower circulating triglyceride and fatty acid levels, and lower mortality. AZIP characteristics that are more severe on the B6 background include increased liver size and liver triglyceride content. A unifying hypothesis is that the B6 strain has higher triglyceride clearance into the liver, with lower triglyceride levels elsewhere. This may account for the observation that the B6 AZIP mice have less insulin-resistant muscles and more insulin-resistant livers, than do the FVB AZIP mice. B6 wild type, as well as B6 AZIP, mice have increased triglyceride clearance relative to FVB, which may be explained in part by higher serum lipase levels and liver CD36 fatty acid translocase mRNA levels. Thus, it is likely that increased triglyceride clearance in B6, as compared with FVB, mice contributes to the strain differences in insulin resistance and lipid metabolism.

Diabetes, obesity, and atherosclerosis are examples of complex genetic diseases (1, 2). They are caused by interactions among many genes and environmental factors. Each susceptibility gene is thought to make a relatively small contribution to the phenotype and it is likely that different populations carry overlapping, but distinct sets of susceptibility genes. In humans, a number of studies have identified chromosomal regions that contain diabetes or obesity loci, but the specific gene and responsible mutations/polymorphisms have been definitively identified in only a few cases (3).

A number of major diabetes and obesity genes have been identified in mice. Indeed, our current knowledge of the physiology of body weight regulation and obesity is the direct result of the study of mutant mice. Whereas most of the genes discovered cause monogenic disease, they identify the pathways that are likely to be affected in polygenic disease. Additionally, the background genotype has a profound effect on the phenotype of monogenic mutants. For example, homozygous ob or db (now officially Lepo and Leprdb, affecting leptin and leptin receptor, respectively) mutations on the B6 background cause obesity, mild hyperglycemia, and profoundly elevated insulin levels. The same mutations on the C57BLKS/J background produce severe diabetes and early mortality, with less obesity and hyperinsulinemia (4, 5).

We have been studying the transgenic A-ZIP/F-1 (hereafter, AZIP) mouse, which was produced by adipose-selective expression of a dominant-negative protein (6). These mice lack virtually all of their white adipose tissue (WAT). The lack of WAT causes severe insulin resistance, hyperglycemia, elevated serum triglycerides and fatty acids, and massively increased liver triglyceride levels (6–9). The syndrome is remarkably similar to that of patients with severe lipoatrophic diabetes (10, 11).

To date, studies of the AZIP mice have used a pure FVB genetic background. Here we report the characterization of the AZIP phenotype on the B6 background and compare it to the FVB phenotype. The FVB and B6 backgrounds were chosen for practical reasons. The original AZIP transgenic mouse was generated on the FVB background, which, for technical ease, is commonly used to make transgenic mice (12). The other strain, B6, is arguably the best characterized mouse strain, particularly for metabolic phenotypes, and is among the first to have its genome sequenced. The FVB and B6 strains are genetically quite distant (13).

Here we report that genetic background has a profound effect on many aspects of the AZIP phenotype, including opposite effects on muscle and liver insulin resistance. Furthermore, we have observed differences in triglyceride clearance between wild type FVB and B6 mice that may explain some of the AZIP differences.

EXPERIMENTAL PROCEDURES

Mice—All AZIP mice were studied as hemizygotes, produced by breeding hemizygous males with wild type females. B6 AZIP mice were obtained by at least 13 generations of backcrossing AZIP males with B6 (Jackson Laboratory, Bar Harbor, ME) females. Although referred to as B6, all of the B6 AZIP and control males have the FVB Y chromosome. The B6 AZIP mice may carry advantageous FVB alleles, selectively retained during the backcrossing, because some AZIP mice die before reaching breeding age (see “Results”). FVB mice were originally obtained from the Veterinary Resources Program, the National Institutes of Health, and Harlan Sprague-Dawley. Mice were typically housed 3–5

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1 The abbreviations used are: AZIP, A-ZIP/F-1; B6, C57BL/6J; FVB, FVB/N; WAT, white adipose tissue; PPAR, peroxisome proliferator-activated receptor; SREBP-1, sterol response element-binding protein 1; ANOVA, analysis of variance.

3992 This paper is available on line at http://www.jbc.org
Effect of Genetic Background on Lipotoxic Phenotype

The body mass index (BMI) is the body weight/length squared. Organs weights are expressed as a percentage of the body weight. Statistical significance is from two-way ANOVA; single symbols are used for \( p \leq 0.05 \) and double symbols for \( p \leq 0.005 \); \( \dagger \) refers to FVB versus B6; and \( \dagger \dagger \) refers to interaction between transgene status and background genotype. Muscle triglyceride (TG) in wild type (but not AZIP) mice is unreliable due to the presence of adipose tissue (Ref. 14 and O. Gavrilova, unpublished observations).

### Table I

**Anatomical characterization of male AZIP mice at 28 weeks of age**

| Statistics | FVB WT | FVB AZIP | FVB: AZIP/WT | B6 WT | B6 AZIP | B6: AZIP/WT |
|------------|--------|----------|--------------|-------|---------|-------------|
| n = 9      |       |          |              | n = 9 |         | n = 9       |
| Body weight (g) | ⌧\( \dagger \) | 33.1 ± 1.2 | 31.2 ± 0.9 | 94    | 38.4 ± 1.7 | 31.5 ± 0.7  |
| Body length (cm) ** | 8.20 ± 0.06 | 8.05 ± 0.11 | 98    | 7.86 ± 0.12 | 7.74 ± 0.10 | 98          |
| BMI (g/cm\(^2\)) \( \dagger \dagger \) \( \dagger \dagger \) | 0.492 ± 0.013 | 0.481 ± 0.009 | 96    | 0.620 ± 0.031 | 0.526 ± 0.011 | 85          |
| BAT weight (%) \( \dagger \dagger \) \( \dagger \dagger \) \( \dagger \dagger \) | 0.62 ± 0.04 | 0.08 ± 0.01 | 14    | 0.41 ± 0.03 | 0.08 ± 0.01 | 20          |
| Hepatic weight (%) \( \dagger \dagger \) \( \dagger \dagger \) \( \dagger \dagger \) | 0.47 ± 0.07 | 0.57 ± 0.02 | 121   | 0.57 ± 0.02 | 0.49 ± 0.01 | 131         |
| Spleen weight (%) \( \dagger \dagger \) \( \dagger \dagger \) \( \dagger \dagger \) | 0.38 ± 0.02 | 0.83 ± 0.17 | 221   | 0.27 ± 0.01 | 0.53 ± 0.07 | 198         |
| Kidney weight (%) \( \dagger \dagger \) \( \dagger \dagger \) \( \dagger \dagger \) | 1.48 ± 0.06 | 2.00 ± 0.07 | 135   | 1.10 ± 0.04 | 1.22 ± 0.03 | 111         |
| Liver weight (%) \( \dagger \dagger \) \( \dagger \dagger \) \( \dagger \dagger \) | 4.1 ± 0.2 | 9.1 ± 0.2 | 223   | 4.4 ± 0.2 | 19.3 ± 1.8 | 435         |
| Liver TG (μmol/g) \( \dagger \dagger \) \( \dagger \dagger \) \( \dagger \dagger \) | 14 ± 2 | 166 ± 44 | 38 ± 4 | 295 ± 24 |           |             |
| Muscle TG (μmol/g) \( \dagger \dagger \) \( \dagger \dagger \) \( \dagger \dagger \) | 11.1 ± 0.6 | | | | | |

Per cage, on a 12-h light/dark cycle (0600–1800), and fed NIH-07 rodent chow (12.9 kcal % fat, Zeigler Brothers Inc., Gardners, PA) and water ad libitum. Wild-type controls were generally littermates. Animal experiments were approved by the NIDDK Animal Care and Use Committee.

Biochemical Assays—Blood was obtained by tail bleed from unanesthetized mice and was immediately analyzed for glucose concentration. Glycemia was measured using a Glucose-Elite (Bayer Corp., Elkhart, IN). Triglycerides (number 339-11; Sigma), nonesterified fatty acids (number 1383175; Roche Molecular Biochemicals), cholesterol (number 401; Sigma), and insulin (number SRI-13K; Linco Research Inc., St. Charles, MO) were assayed according to the assay suppliers’ procedures.

Liver triglycerides were measured as follows (14). Tissue (~100 mg) was homogenized in 3 ml of 2:1 chloroform:methanol and shaken at room temperature for 4 h. Next, 1.5 ml of 0.9 M NaCl was added, mixed, centrifuged, and the lower organic phase containing triglycerides was transferred to a new glass tube and air-dried. The residue was hydrolyzed (70 °C, 1 h) in 200 μl of 3 M KOH in 65% ethanol and the released glycerol was quantitated using a radiometric glycerol kinase assay (15, 16). The radiometric assay is more sensitive and accurate than the colorimetric assay.

Euglycemic-Hyperinsulinemic Clamps—The clamp protocols are based on those of Kim and Shulman (17–20) and have been described in detail. The target plasma insulin level was 4 ng/ml and the target plasma glucose was 110–190 mg/dl.

In Vivo Triglyceride Studies—Triglyceride secretion was studied by measuring the increase in circulating triglycerides after lipase inhibition by WR1339 (21, 22). Briefly, mice were given ad libitum access to a fat-free diet (Frosted Flakes, Kellogg, Battle Creek, MI) for 4 h then anesthetized with ketamine (100 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (10 mg/kg; Phoenix Scientific, St. Joseph, MO), and WR1339 (Sigma, number T-8761, 100 mg of a 1:10 dilution in phosphate-buffered saline) was injected via warmed tail vein and blood samples were withdrawn at 0, 1, and 2 h. Plasma triglycerides were measured colorimetrically. Data are expressed as milligrams of triglyceride/kg of body weight/ℎ, assuming plasma volume is 3.5% of body weight.

Clearance of triglyceride (400 μl of peanut oil, delivered by gavage) from the circulation was measured in mice previously fasted for 4 h. Blood was taken hourly via tail vein for 6 h and plasma triglycerides were measured as described above.

Northern Blot Hybridization of RNA—Total RNA was prepared, hybridized, and quantitated by PhosphorImager as described previously (9). Additional cDNA probes were prepared by reverse transcription–PCR using the following primers (5’ primer listed first in each case). Acetyl-CoA carboxylase, 5’-GGGACTCTCATGACCAGGACAGG and 5’-CTGTGACTTGCGAGGTCAGAGG; SREBP2, 5’-CCCGTGGTACCATGTGTGTGCGG and 5’-CTGACCGTTGCGAGGTCAGAGG. A cDNA probe for glucose-6-phosphatase (23) was excised from plasmid DNA.

Statistical Analysis—Results are reported as mean ± S.E. Data were analyzed using SigmaStat (SPSS Inc., Chicago, IL) by t test or two-way ANOVA followed by the Tukey test for pairwise multiple comparisons.

### RESULTS

**B6 AZIP Mice Have Virtually No White Adipose Tissue**—To follow up on preliminary observations suggesting an effect of genetic background on the AZIP phenotype, we quantitated multiple characteristics of the AZIP mice. No significant white adipose depots were found in AZIP mice on either the B6 or FVB genetic background. The brown adipose tissue phenotype of the B6 AZIP and FVB AZIP mice was also similar, with relatively normal amounts and appearance at birth followed by reduction in size (Table I) and an inactive gross and histological morphology in adulthood (data not shown).

### Changes in Body Weight and Blood Chemistries—FVB AZIP mice are the same size at birth as wild type littermates, but rapidly fall behind in growth, later catching up (males) or growing larger (females) than their littermates (6). B6 AZIP males have a different growth pattern, remaining smaller than their littermates (Fig. 1). The growth patterns of B6 AZIP and FVB AZIP females are comparable (data not shown).

FVB AZIP mice are severely hyperglycemic, averaging 542 mg/dl in males, compared with 164 mg/dl in controls (Fig. 1). In marked contrast, male B6 AZIP mice have a transient hyperglycemia, peaking at ~300 mg/dl at 8–12 weeks of age, followed by near normal glucose levels thereafter. The blood glucose levels in female B6 AZIP mice averaged 60 mg/dl higher than the wild type controls and did not show the multiphasic pattern of the male AZIP mice (data not shown). Serum insulin levels were greatly and similarly elevated in B6 and FVB AZIP mice (Fig. 1). These data suggest that the lower, more normal glucose levels in B6 AZIP mice are not because of more insulin secretion, but rather to less insulin resistance.

Serum triglyceride levels are also affected by background genotype (Fig. 1). Wild-type male FVB mice had higher triglyceride levels (172 mg/dl) than B6 mice (91 mg/dl). In the FVB mice the AZIP transgene caused a remarkable increase in the triglyceride levels (to ~750 mg/dl in young mice (Fig. 1). In the decrease in triglycerides with aging was not seen in other mice at up to 17 weeks of age). In contrast, in male B6 AZIP mice, serum triglyceride levels averaged only 121 mg/dl. Female B6 wild type and AZIP mice had triglyceride levels of 59 and 51 mg/dl, respectively. Thus, the AZIP transgene barely affects the B6 triglyceride and AZIP triglyceride levels, but causes a remarkable increase in FVB triglyceride levels.
Serum fatty acid levels were similar in B6 wild type and AZIP mice. Fatty acid levels in FVB wild type and AZIP mice were higher than in B6 mice, with the younger FVB AZIP mice tending to be higher than the wild type FVB mice (Fig. 1).

Taken together, these data demonstrate no background genotype effect on serum insulin levels, but appreciable background genotype effects on body weight, blood glucose, serum triglyceride, and fatty acid levels. In each case the B6 background gives a milder phenotype.

To see if the biochemically milder phenotype affects clinical outcome, survival was scored, and indeed was markedly greater in the B6 AZIP mice. At weaning (n = 292) B6 AZIP male and female mice were present at 60 and 96% of the expected levels, respectively. For comparison, FVB AZIP male and female mice were present at only 36 and 47% of the expected levels, respectively (6). Mortality between weaning and 6 months of age was also less in the B6 than the FVB AZIP mice, although this was not studied quantitatively.

**Tissue Insulin Sensitivity in B6 and FVB AZIP Mice**—When AZIP mice were studied anatomically, the B6 AZIP mice showed remarkably greater hepatomegaly (4.4-fold enlarged in males and 5.2-fold in females) than did the FVB AZIP mice (2.2-fold in males) (Table I, Fig. 2). (The weights of other organs, brown adipose tissue, heart, spleen, and kidney, also showed significant effects of background genotype, with the AZIP changes generally greater on the FVB background, Table I.) The increased liver weight reflected massively increased liver triglyceride content, giving the livers a lighter color and characteristic histology. Interestingly, in the wild type mice liver triglyceride content was also greater (~3-fold) in the B6 background.

Because organ triglyceride content correlates with insulin resistance, euglycemic-hyperinsulinemic clamps were performed to evaluate the individual tissue insulin responsiveness of the AZIP mice. Wild-type B6 and FVB mice were similar in all clamp parameters whereas the B6 AZIP and FVB AZIP mice were quite different (Table II, Fig. 3). The endogenous glucose production of the FVB AZIP mice was suppressed almost as well as in the wild type FVB mice (53 versus 69%, p = nonsignificant; in other studies the difference reached significance (24)). However, the endogenous glucose production in B6 AZIP mice did not suppress at all during the clamp (Table II, Fig. 3A). Because endogenous glucose production is chiefly a measure of liver glucose production, these data demonstrate that the B6 AZIP mice have more severe liver insulin resistance than do the FVB AZIP mice.

During the clamp, whole body glucose uptake in FVB AZIP mice was only 32% of wild type controls, indicating severe muscle insulin resistance (Fig. 3B). In contrast, B6 AZIP whole body glucose uptake in mice was 66% of control levels. Direct measurement of muscle glucose uptake with [14C]2-deoxyglucose gave similar results: in FVB AZIP mice the rate was 22% of FVB wild type and in B6 AZIP mice it was 63% of B6 wild type (Fig. 3C). This pattern was also observed for whole body and muscle glycolysis and for glycogen synthesis (Table II). Thus, the muscles of the FVB AZIP mice are more insulin resistant than those of the B6 AZIP mice, the opposite of the pattern observed for liver.

**Lipid Metabolism**—Liver triglyceride levels were determined by triglyceride synthesis, uptake, and secretion rates. To quantitate triglyceride secretion, the rate of increase in circulating triglycerides was measured after inhibiting clearance with Triton WR1339 (21). Wild-type B6 and FVB mice had similar secretion rates, which were increased significantly by the FVB AZIP mice.

Triglyceride clearance was studied by measuring serum triglyceride levels after an oral lipid load. In wild type FVB mice, serum triglycerides peaked at 2 h (382 mg/dl) and then decreased gradually (Fig. 4B). The increase was accentuated (980 mg/dl) and occurred later (4 h) in FVB AZIP mice, demonstrating that triglyceride clearance on FVB background is greatly impaired in the absence of WAT. Surprisingly, a totally different result was found for the B6 mice. Serum triglycerides rose only minimally in wild type and transgenic mice, peaking at 82 and 128 mg/dl in B6 wild type and B6 AZIP mice, respectively. This was not because of poor triglyceride uptake from the intestine in B6 mice because, in the absence of triglyceride clearance, serum triglyceride levels increased equally in B6 and FVB mice after an oral lipid load (Fig. 4C). Thus, B6 mice show much more efficient clearance of circulating triglyceride than do FVB mice.

Increased lipase activity should increase triglyceride clearance. Serum lipase activity (without heparin), which in mice is

**Fig. 1. Growth and circulating glucose, insulin, triglyceride, and fatty acid levels in male AZIP mice.** Symbols are: B6 wild type (○), B6 AZIP (●), FVB wild type (■), and FVB AZIP (■). Samples are from longitudinal follow up of single cohorts of mice, and were obtained from unanesthetized mice in the nonfasting state, between 0900 and 1200. Note the logarithmic scale for the insulin data. Data are mean ± S.E., n = 4–9/group. Averages of all glucose values in mg/dl are (mean ± one S.D.): B6 wild type, 154 ± 18, n = 123; FVB wild type, 164 ± 23, n = 81; FVB AZIP, 542 ± 104, n = 56. Averages of all triglyceride values in mg/dl are: B6 wild type, 91 ± 26, n = 47; B6 AZIP, 120 ± 60, n = 50; FVB wild type, 172 ± 87, n = 72.
lately a measure of hepatic lipase (25), was higher in B6 than FVB mice (Table III). Post-heparin plasma showed modest increases in both hepatic and lipoprotein lipase activity in wild type B6 as compared with FVB mice, but no striking effect of the AZIP transgene (Table III). Hepatic lipase mRNA levels were not different between the groups, whereas endothelial lipase mRNA levels were lower in the wild type FVB livers than in the other groups (Fig. 5). Liver lipoprotein lipase mRNA levels were <1/20 the level of adipose tissue levels, too low to determine whether differences exist between the groups (data not shown). These data suggest that increased lipase activity may contribute to the increased triglyceride clearance.

Gene Expression.—Liver mRNA levels were measured in the B6 and FVB strains (Fig. 5). There was a significant background genotype effect on mRNA levels of lipogenic genes (LXRα, SREBP1, PPARγ, acyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase I), fatty acid oxidation genes (acyl-CoA oxidase and carnitine palmitoyltransferase I), and gluconeogenic genes (glucose-6-phosphataseae and fructose-1,6-bisphosphatase), with the B6 levels typically being 2–3-fold higher than the FVB levels. Comparing AZIP to wild type mice, the lipogenic gene (SREBP1, PPARγ, acyl-CoA carboxylase, fatty acid synthase, and SCD-1) mRNA levels were higher in the B6 mice.

FIG. 2. Liver appearance, histology, weight, and triglyceride content and muscle triglyceride levels at 28 weeks of age. In each pair of the indicated background genotype and sex, the wild type control is in the left bar and the AZIP in the right bar. Data are mean ± S.E., n = 5–9/group, n = 4/group for muscle triglyceride.

**Table II**

Euglycemic-hyperinsulinemic clamp measurements

|Statistics | FVB WT | FVB AZIP | B6 WT | B6 AZIP |
|-----------|--------|----------|-------|---------|
|Body weight (g) | * | 20.9 ± 0.7 | 21.3 ± 1.0 | 19.2 ± 0.6 | 19.1 ± 0.5 |
|Basal blood glucose (mg/dl) | ‡‡ * ++ | 144 ± 14 | 254 ± 15 | 160 ± 11 | 175 ± 9 |
|Basal insulin (ng/ml) | ‡‡ | 0.9 ± 0.1 | 1.0 ± 0.1 | 0.6 ± 0.03 | 0.8 ± 0.1 |
|Clamp blood glucose (mg/dl) | ‡‡ | 111 ± 7 | 110 ± 3 | 114 ± 5 | 128 ± 4 |
|Clamp insulin (ng/ml) | ‡‡ | 3.2 ± 0.5 | 3.3 ± 0.4 | 3.1 ± 0.3 | 3.0 ± 0.4 |
|Glucose infusion rate (µmol/kg BW/min) | ‡‡ | 235 ± 31 | 41 ± 9 | 294 ± 21 | 67 ± 23 |
|Whole body glycolysis (µmol/kg BW/min) | ‡‡ * | 237 ± 42 | 76 ± 9 | 279 ± 21 | 182 ± 17 |
|Whole body glycogen synthesis (µmol/kg BW/min) | ‡‡ | 38 ± 11 | 23 ± 7 | 42 ± 10 | 35 ± 16 |
|Muscle glycolysis (µmol/kg muscle/min) | ‡‡ + | 213 ± 21 | 84 ± 13 | 202 ± 10 | 160 ± 27 |
|Muscle glycogen synthesis (µmol/kg muscle/min) | ‡‡ | 31 ± 5 | 7 ± 1 | 24 ± 4 | 16 ± 5 |
|Suppression of EGP (%) | ‡‡ * ++ | 69 ± 13 | 53 ± 5 | 81 ± 11 | –8 ± 9 |

Unexpectedly, a huge, 20-fold elevation in the hepatic CD36 mRNA level was observed in B6 wild type as compared with FVB wild type mice (Fig. 6, **top**). The AZIP mice on both genetic backgrounds had high CD36 mRNA levels, similar to wild type B6, but 15–25-fold greater than wild type FVB, mice. In contrast, CD36 mRNA levels in muscle were not different between wild type and AZIP mice on either the FVB or B6 genetic backgrounds. Taken together, these results demonstrate that
there is a liver-specific difference in the regulation of the CD36 gene between the wild type FVB and B6 lines.

As an additional test of the contribution of CD36 to circulating triglyceride levels, liver CD36 mRNA and serum triglyceride levels were measured in four more strains (SWR/J, C3H/HeJ, BALB/cJ, and 129/SvJ) and repeated in the B6 and FVB mice (Fig. 6, bottom). There is an approximate inverse correlation between serum triglycerides and liver CD36 mRNA levels, but the CD36 levels cannot completely account for the variation in serum triglycerides among the six strains.

DISCUSSION

The AZIP Phenotype Depends on the Genetic Background—The AZIP phenotype on the B6 background is generally less severe than that on the FVB background. Specifically, the B6 AZIP mice have less hyperglycemia, lower circulating triglyceride and fatty acid levels, faster triglyceride clearance, lower mortality, and less muscle insulin resistance. Features of the AZIP phenotype that are similar on both backgrounds include the severe lack of WAT, hugely increased insulin levels, and increased triglyceride secretion. Characteristics of the AZIP phenotype that are more profound on the B6 background include hepatic insulin resistance and steatosis.

Previous studies of the effect of genetic background on diabetes using ob/ob and db/db mice found β-cell failure causing premature death on the C57BLKS/J (26) and CBA/LtJ (27) backgrounds. In contrast, on the B6 (28), 129/J (29), and FVB (30) backgrounds, the same mutations caused more obesity but milder hyperglycemia, because of increased insulin production, with β-cell hypertrophy and hyperplasia. Thus the studies of ob/ob, db/db, and AZIP mice agree, suggesting that the B6 and FVB backgrounds have robust islets, carrying alleles that promote β-cell production, longevity, and/or insulin secretion. Because the FVB and B6 strains differ in their insulin resistance phenotypes and because the confounding effects of β-cell failure do not complicate the studies, these strains can be used to identify insulin resistance modifier genes, for example, by the quantitative trait locus approach.

How Does Lipoatrophy Cause Diabetes?—The lack of adipose tissue is the primary cause of the metabolic symptoms known as lipoatrophic diabetes as shown by the correlation between
Effect of Genetic Background on Lipoatrophic Phenotype

TABLE III
Serum chemistries of male mice

| Statistics | FVB WT | FVB AZIP | B6 WT | B6 AZIP |
|------------|--------|----------|-------|---------|
| Glucose (mg/dl) | $\ddagger\ddagger$ ** ++ | 178 ± 11 | 713 ± 87 | 208 ± 16 | 225 ± 27 |
| Triglyceride (mg/dl) | $\ddagger\ddagger$ ** | 135 ± 18 | 278 ± 115 | 79 ± 8* | 108 ± 13 |
| Cholesterol (mg/dl) | $\ddagger\ddagger$ ** ++ | 117 ± 5 | 124 ± 28 | 129 ± 12 | 245 ± 22 |
| Albumin (g/dl) | $\ddagger$ ** | 2.8 ± 0.2 | 3.1 ± 0.2 | 3.9 ± 0.1 | 4.5 ± 0.1 |
| Protein (g/dl) | $\ddagger\ddagger$ ** | 4.8 ± 0.1 | 5.4 ± 0.3 | 5.5 ± 0.2 | 6.8 ± 0.2 |
| BUN (mg/dl) | ** | 39 ± 5 | 52 ± 13 | 29 ± 1 | 28 ± 1 |
| ALT (units/liter) | $\ddagger$ ** | 12 ± 1 | 72 ± 17 | 27 ± 12 | 265 ± 48 |
| AST (units/liter) | $\ddagger\ddagger$ ++ | 148 ± 31 | 146 ± 35 | 81 ± 16 | 275 ± 41 |
| Lactate dehydrogenase (units/liter) | $\ddagger\ddagger$ ** | 726 ± 163 | 1003 ± 161 | 440 ± 142 | 2550 ± 451 |
| Alkaline phosphatase (units/liter) | $\ddagger\ddagger$ ** | 43 ± 3 | 148 ± 32 | 87 ± 14 | 232 ± 25 |
| Creatine kinase (units/liter) | ** | 18 ± 2 | 40 ± 11 | 175 ± 61 | 232 ± 44 |
| Lipase (units/liter) | ** | 22 ± 1 | 24 ± 2 | 31 ± 2 | 35 ± 4 |
| Postheparin lipoprotein lipase (mmol of fatty acid/ml/h) | * | 38 ± 6 | 45 ± 4 | 64 ± 8 | 54 ± 5 |
| Postheparin hepatic lipase (mmol of FA/ml/h) | ** | 12 ± 1 | 15 ± 2 | 20 ± 1 | 18 ± 1 |

![Fig. 5](image)

Liver mRNA levels of genes involved in metabolism of glucose, triglycerides, and fatty acids. In each group, the order of the bars is FVB wild type, FVB AZIP, B6 wild type, and B6 AZIP. mRNA levels were measured by Northern blotting in livers from non-fasting mice euthanized between 0900 and 1200. Data are normalized with FVB wild type levels = 100. Data are from male mice, age 28 weeks (mean ± S.E., n = 4–6/group). Statistical significance via two-way ANOVA: single symbols indicate $p \leq 0.05$ and double symbols for $p \leq 0.005$; $\ddagger$ refers to AZIP versus wild type and the single asterisk (*) refers to FVB versus B6. No significant interaction between transgene status and background genotype was detected. ACC, acetyl-CoA carboxylase; G6P, glucose-6-phosphatase; FAS, fatty acid synthase; CPT1, carnitine palmitoyltransferase 1; PEPCK, phosphoenolpyruvate carboxykinase; IRS-2, insulin receptor substrate 2; AOX, acyl-CoA oxidase; LXRa, liver X receptor α; SCD-1, stearoyl-CoA desaturase 1; F1-6BP, fructose-1.6-bisphosphatase.

the degree of fat loss and syndrome severity and by the reversal of all features by adipose tissue transplantation (8). Adipose tissue ablation removes triglyceride storage capacity and adipose hormones such as leptin and adiponectin/Acrp30. The lack of fat is signaled to the liver, causing an increase in hepatic fatty acid synthesis, triglyceride storage, and secretion. The fatty liver can be thought of as an appropriate compensatory response by the body to the deficit of adipose tissue triglyceride. The changes in liver physiology are accomplished through increased levels of the transcription factors sterol element-binding protein 1c (SREBP-1c) (31) and peroxisome proliferator-activated receptor-γ (PPARγ) (9). SREBP-1c increases lipid accumulation in hepatocytes (32) and livers of mice (33, 34) and may act by increasing PPARγ expression (35). Recent experiments demonstrate that liver-specific ablation of PPARγ greatly reduces hepatic triglyceride levels in the AZIP mice, suggesting a role for PPARγ in driving hepatic steatosis.2

The nature of the signals from WAT to the rest of the body, and the route they take are not known. Leptin deficiency clearly contributes to the insulin resistance and lipid abnormalities (36–39). Leptin may be acting via the hypothalamus, elsewhere in the brain, and/or directly on tissues such as liver and muscle (40, 41). Adiponectin/Acrp30 (42–44) deficiency does not appear to be the major cause of the diabetes of the B6; and AZIP metabolic phenotype focuses on hepatic triglyceride and lipid clearance. The B6 mice have higher serum lipase levels and better clearance of an oral lipid load. Presumably, more of the lipid ends up in the liver in the B6 mice, whereas slightly less ends up in the muscle. The increased liver triglyceride causes increased hepatic insulin resistance, whereas the reduced muscle triglyceride levels lead to less muscle insulin resistance. Increased hepatic lipogenesis may also contribute. Previous reports have correlated muscle triglyceride levels with muscle insulin resistance (45, 46), liver insulin resistance with liver triglyceride content (47), and improvements in one at the expense of the other (48, 49). However, the tissue triglycerides themselves are probably not the ultimate cause of decreased insulin signaling, but are rather a surrogate for molecules as yet unidentified (50).

2 O. Gavrilova, M. Haluzik, J. J. Cutson, L. Johnson, K. R. Dietz, C. Nicol, C. Vison, F. Gonzalez, and M. L. Reitman, submitted for publication.
and we believe that the insights are generally applicable. For example, treatment with leptin improves the diabetes and lipid abnormalities of both mice (36, 39) and humans with lipoatrophy (51). The differences between the FVB and B6 backgrounds is also relevant, suggesting that human genetic heterogeneity greatly influences the observed lipoatrophic phenotype.

Lipoprotein physiology is somewhat different between mice and humans. Mice have higher high density lipoprotein, lower low density lipoprotein, and are resistant to atherosclerosis. Thus to study atherosclerosis in the mouse, a high cholesterol/high fat diet and/or Ldlr−/− or Apoe−/− mutations are usually used to accelerate the process (52). The B6 background is more susceptible to atherosclerosis than other strains and B6 mice have the lowest circulating triglyceride levels of 17 mouse strains tested (53). Indeed, a direct comparison of ApoE-deficient mice on the B6 and FVB backgrounds found the B6 mice to have lower total cholesterol, high density lipoprotein cholesterol, ApoA-I, and ApoA-II levels and larger atherosclerotic lesions (54). It is possible that mechanistic understanding of the increased triglyceride clearance will shed light on the B6 predisposition to atherogenesis. Possibly lipid uptake into other tissues is also facilitated in the B6 strain. The FVB to B6 comparison points to triglyceride clearance as a modifier of the diabetes and lipid phenotypes.

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