The *db/db* Mouse, a Model for Diabetic Dyslipidemia: Molecular Characterization and Effects of Western Diet Feeding

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Diabetic dyslipidemia is a major factor contributing to the accelerated atherosclerosis in type 2 diabetes mellitus. Although several mouse models are available, the plasma lipoproteins in response to diet have not been fully characterized in these animals. In this study, we have characterized the plasma lipoproteins and related apolipoproteins, as well as the vascular lipases, in *diabetes* (*db/db*) mice and their nondiabetic controls (+/?) in the C57BL/KsJ strain. Within 6 weeks of age, *db/db* mice developed significant obesity, fasting hyperglycemia, and hyperinsulinemia. By FPLC analysis, *db/db* mice showed a prominent peak in the low-density lipoprotein (LDL) range that was absent in +/? mice, although high-density lipoprotein (HDL) was the predominant species in both groups of animals. Postheparin lipoprotein lipase (LPL) activity in *db/db* mice was 28% of the level in +/? mice. Upon feeding a human-like 0.15% (wt/wt) cholesterol and 21% (wt/wt) fat "Western" diet, *db/db* mice developed elevated plasma cholesterol, accompanied by an exaggerated apolipoprotein E (apoE) response compared with +/? mice. FPLC analysis showed that the marked hypercholesterolemic response in *db/db* mice was the result of a massive increase in the LDL region, which overshadowed a moderate increase in HDL. We next isolated lipoproteins by ultracentrifugation and characterized them by nondenaturing gradient gel electrophoresis. With regular chow, *db/db* mice had almost exclusively small dense LDL with a peak size at 21.4 nm, as compared with 26.6 nm in nondiabetic controls. On the Western diet, the small dense LDLs persisted but larger particles also appeared in *db/db* mice, whereas the size distribution in +/? mice was unchanged by the diet. Our results suggest that *db/db* mice fed a Western diet have a plasma lipoprotein phenotype that shows some similarities to that in patients with type 2 diabetes mellitus, and that *db/db* mice are a useful model to study the pathogenesis and treatment of diabetic dyslipidemia.

CARDIOVASCULAR DISEASE is the most frequent cause of morbidity and mortality in patients with type 2 diabetes mellitus.[1,2] Diabetic dyslipidemia is thought to be a major factor in the accelerated atherosclerosis associated with type 2 diabetes.[3-5]

Dyslipidemia in type 2 diabetes is associated with elevated very–low-density lipoprotein (VLDL) and triglyceride and low high-density lipoprotein (HDL).[3,4,6] Plasma low-density lipoprotein (LDL) has been reported as normal[7,8] or elevated.[9] Importantly, there appears to be an increased proportion of small dense LDL.[10-16] While there are metabolic studies in humans exploring the various lipoprotein abnormalities in diabetes[17,18] (reviewed in various publications[3,4,6,19,20]), the identification of appropriate diabetic animal models that develop dyslipoproteinemia will provide an important tool for studies on the pathogenesis and treatment of diabetic dyslipidemia.

The mouse has become a popular model for various dyslipidemias in the last few years because it is genetically well defined and there is an abundance of natural mutations, as well as targeted mutations created in the laboratory.[21-24] A number of extant single-gene mutations resulting in obesity in rodents have been cloned. These mutations are yellow, obese, diabetes, fat, and tubby.[25] The *db* mutation was traced to a mutation in the leptin receptor.[26] Leptin receptor mutations were also identified in 2 other grossly obese rodents, corpulent and fatty Zucker rats.[27,28] The plasma lipids of a number of genetic models of type 2 diabetes have been measured. Of these, the *db/db* mouse appears to have the highest plasma triglyceride and cholesterol,[29] and may be a good model for diabetic dyslipidemia. However, to date, the lipoprotein abnormality in *db/db* mice is poorly characterized and the underlying molecular pathology is essentially unexplored. In this report, we present a detailed characterization of the lipoprotein changes in *db/db* mice at the molecular level, and the effect of a human-like Western diet[30] on the lipoprotein phenotype in *db/db* mice.

MATERIALS AND METHODS

Materials

Total cholesterol, triglyceride, and glucose assay kits, heparin, phosphatidylcholine, triolein, rat serum, and bovine serum albumin were obtained from Sigma Diagnostics (St Louis, MO). [α-^{32}P]dCTP (3,000 Ci/mmol), an insulin assay kit, and Universol were from ICN Pharmaceuticals (Costa Mesa, CA). [α-^{32}P]UTP (800 Ci/mmol) and the random primer labeling kit were obtained from Amersham (Arlington Heights, IL). Enzymes and buffers were obtained from Life Technologies (Grand Island, NY). All other reagents were from Fisher Scientific (Pittsburgh, PA).

Animals and Diets

Homozygous diabetic (*db/db*) mice and nondiabetic control littermates (+/?) of the C57BL/KsJ strain were purchased from the Jackson Laboratory (Bar Harbor, ME) and maintained on a 12-hour light/dark cycle with free access to food and water. Age-matched *db/db* and +/? male mice were fed either a standard chow diet (Harlan Tekland Premier Laboratory Diets, Madison, WI) or a "Western" high-fat diet containing 0.15% (wt/wt) cholesterol and 21% (wt/wt) fat[30] (Harlan Tekland Premier Laboratory Diets).

All animal procedures were performed according to the guidelines of the Animal Review Committee of Baylor College of Medicine.

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Plasma Lipid and Lipoprotein Analyses

After a 5-hour fast, mice were anesthetized by Metofane (Schering-Plough Animal Health, Union, NJ) inhalation and blood samples were obtained from the retro-orbital venous plexus directly into tubes containing EDTA (final concentration, 0.12%). After centrifugation, the plasma was adjusted to 0.05% NaN_3 and 0.015% phenylmethylsulfonyl fluoride (PMSF) and stored at 4°C. The plasma was assayed for glucose, total cholesterol, and total triglyceride using kits from Sigma Diagnostics. The HDL cholesterol level was measured by a phosphotungstic acid precipitation method using HDL cholesterol reagent from Sigma. For lipoprotein separation, plasma (0.2 mL) collected from individual mice was subjected to fast protein liquid chromatography (FPLC) gel filtration on 2 Superose 6 columns (Pharmacia Biotech, Piscataway, NJ) connected in series.[31] Fifty 0.5-mL fractions were collected. The total cholesterol concentration of each FPLC fraction was measured enzymatically with kits from Sigma.

Plasma Apolipoprotein Quantitation and Western Blot Analysis

Apolipoprotein A-I (apoA-I) and apoE were determined in EDTA-plasma samples by radial immunodiffusion[32] using monospecific rabbit antisera raised against purified apolipoproteins. Plasma apoBs were quantified by sodium dodecyl sulfate (SDS) gel electrophoresis and quantitative densitometric scanning as described previously.[33] Western blot analysis of FPLC fractions was performed as described previously.[34]

Postheparin Lipolytic Activity

Lipolytic activity was measured in postheparin plasma of mice by a previously described method.[35] The *db/db* and +/? mice were injected via the tail vein with heparin (100 U/kg body weight) and blood was collected 5 minutes later, which coincided with peak lipoprotein lipase (LPL) activity. Twenty microliters of plasma was assayed for lipolytic activity in the presence of 400 μL triglyceride emulsion containing 5.0 μCi/mL glycerol tri[9,10-^{3}H]oleate, 0.60 mg/mL phosphatidylcholine, 5.0 mg/mL nonradioactive triolein, 0.11 mol/L NaCl, 5.5 mg/mL albumin, 0.178 mol/L Tris hydrochloride buffer (pH 8.2), and 5% (vol/vol) heat-inactivated rat serum. The reaction mixture was incubated at 37°C for 60 minutes. After incubation, 200 μL of the mixture was extracted with 3.25 mL methanol:chloroform:heptane (1.41:1.25:1.0) and 1.05 mL 50-mmol/L carbonate/borate buffer (pH 10.5). After centrifugation at 2,500 rpm for 20 minutes, 1 mL of the aqueous phase was added to 4 mL Universol and counted in a scintillation counter for 1 minute. Lipolytic activity was also measured in the presence of 1 mol/L NaCl in the reaction mixture; NaCl-inhibited activity was taken as LPL, and resistant activity as hepatic triglyceride lipase (HTGL) activity. Enzyme activity was expressed as units per liter (1 U = 1 μmol free fatty acid released per minute).

RNA Isolation

For the isolation of tissue RNA, mice were killed by cervical dislocation and the tissues were removed and immediately frozen in liquid nitrogen. Total RNA was prepared by homogenizing frozen tissue in Ultraspec RNA (Biotecx Laboratories, Houston, TX). The homogenates were extracted with chloroform according to the product protocol, and total RNA was precipitated with 2-propanol, rinsed twice with 75% ethanol, dried briefly, and dissolved in diethylpyrocarbonate-treated distilled water. Proteinase K (500 μg/mL) treatment, phenol/chloroform extraction, and ethanol precipitation were used for RNA isolation from the small intestine.

Northern Blot Analysis

Twenty micrograms of total RNA was subjected to electrophoresis on a 1% agarose/6% formaldehyde gel in the running buffer (20 mmol/L morpholinepropanesulfonic acid, 5 mmol/L sodium acetate, and 1 mmol/L EDTA). At the end of the electrophoresis, the RNA was transferred to Hybond N+ membranes (Amersham). The membranes were hybridized with ^{32}P-labeled rat apoE (0.4 kb) or mouse HTGL cDNA probes. The same membranes were stripped in boiling 0.1% SDS and then hybridized with a mouse β-actin cDNA probe (pTRI-β-actin-mouse; Ambion, Austin, TX).

RNA Primer Extension Assay for ApoB mRNA Editing

^{32}P-end-labeled mouse apoB primer (BBT9, 5′-AGTCATGTGGATCATAAT-TATCTTTAATATACTGA) was annealed overnight at 45°C with mouse liver total RNA. The annealed products were extended in the presence of 0.5 mmol/L each dATP, dCTP, and dTTP and 0.5 mmol/L dideoxy GTP by the addition of reverse transcriptase (Life Technologies). The primer extension products were resolved on 8% polyacrylamide urea gel electrophoresis, which separated the edited (TAA) and unedited (CAA) bands.[36]

RNase Protection Assay

Antisense RNA probes for mouse apoB, apoE, and LPL cDNA were synthesized using an in vitro transcription kit (Ambion). We performed RNase protection assays using RPA II kit (Ambion) according to the manufacturer's instructions. Protected ^{32}P-RNA bands were visualized by exposure to x-ray film at −80°C with intensifying screens. The band intensities were quantified by exposing the gel to a phosphor screen followed by analysis using a PhosphorImager and the ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Plasma Lipoprotein Isolation and Analysis by Nondenaturing Gradient Gel Electrophoresis

Plasma samples were pooled from 5 +/? mice and *db/db* mice and fractionated by sequential ultracentrifugation at density (d) 1.006 (VLDL), 1.019 (intermediate-density lipoprotein [IDL]), 1.063 (LDL), and 1.21 g/mL (HDL) at 4°C in a type 70.1 Ti rotor (Beckman Instruments, Palo Alto, CA) for 20, 24, 24, and 36 hours, respectively.[37] Collected lipoprotein fractions were dialyzed in 150 mmol/L NaCl, 1 mmol/L EDTA (pH 7.5), 1 mmol/L NaN_3, and 10 μmol/L PMSF. To determine lipoprotein size distribution, LDL and HDL were electrophoresed on 2% to 16% and 4% to 30% nondenaturing polyacrylamide gradient gels, respectively, according to the procedure described by Nichols et al.[38] LDL gels were stained with Oil Red O and HDL gels with Coomassie G250; particle size was assessed by scanning densitometry.

Statistical Analysis

Statistical analyses were performed using the nonpaired Student *t* test with the Sigma Stat program (Jandel Scientific, San Rafael, CA). Results are expressed as the mean ± SD. Statistical significance was defined at a *P* level less than .05.

RESULTS

Body Weight, Blood Glucose, and Insulin in db/db *Mice*

In our initial characterization of the *db/db* mouse as a model for type 2 diabetes mellitus, we evaluated body weight and blood glucose and immunoreactive insulin levels in *db/db* mice and nondiabetic controls from 6 weeks until 15 weeks of age (Fig 1A). The *db/db* mice were 156% of the weight of the nondiabetic mice at 6 weeks. They gained weight rapidly such that at 8 weeks they were 174% of the weight of the controls, increasing further to about 200% of control weight at 14 and 15 weeks. Thus, beginning at an early age, *db/db* mice are grossly obese.

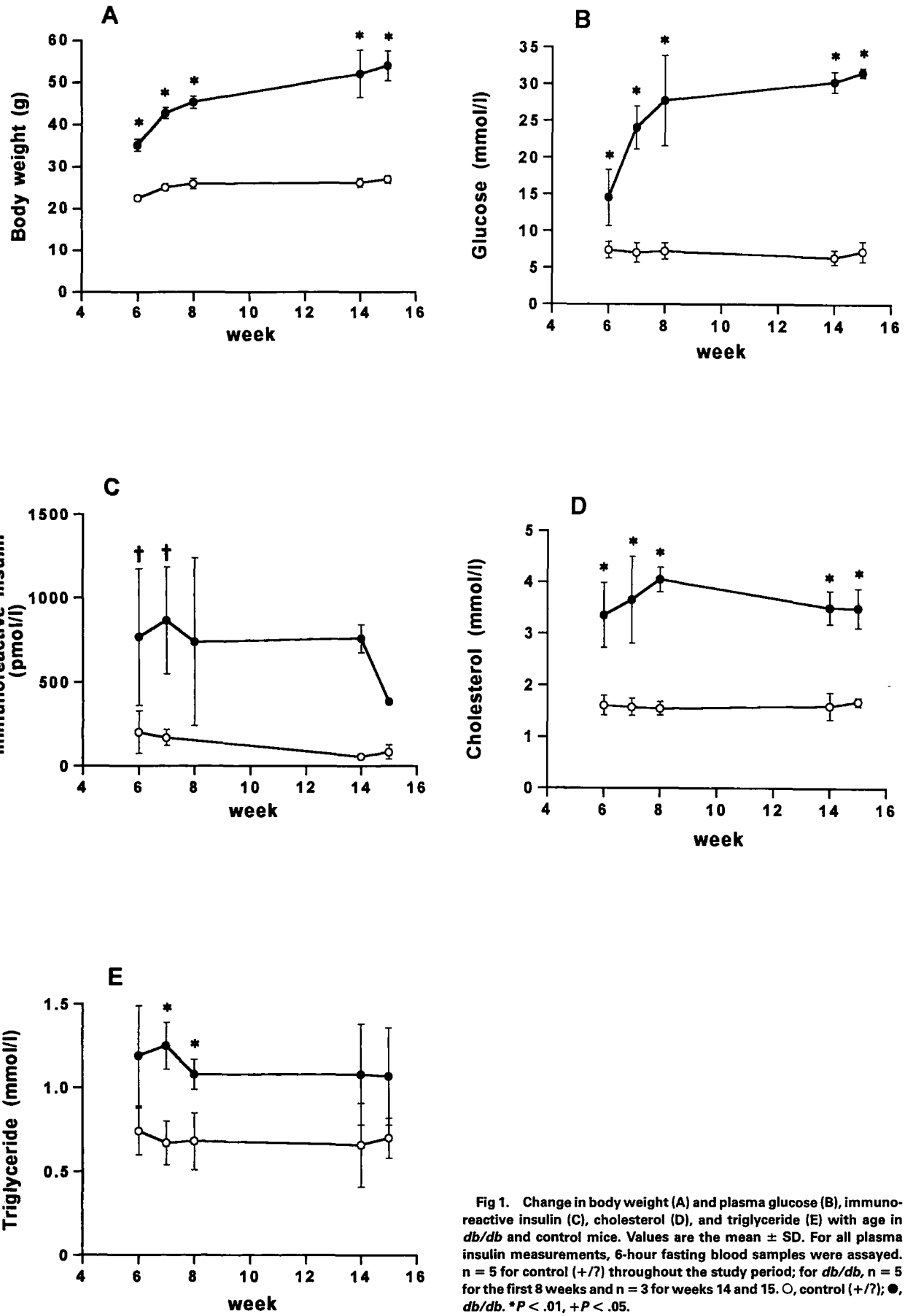

Fig 1. Change in body weight (A) and plasma glucose (B), immunoreactive insulin (C), cholesterol (D), and triglyceride (E) with age in *db/db* and control mice. Values are the mean ± SD. For all plasma insulin measurements, 6-hour fasting blood samples were assayed. n = 5 for control (+/?) throughout the study period; for *db/db*, n = 5 for the first 8 weeks and n = 3 for weeks 14 and 15. ○, control (+/?); ●, *db/db*. *$P < .01$, +$P < .05$.

When we measured the 9 to 10 AM blood glucose concentration after a 6- to 7-hour fast (Fig 1B) at 6 weeks of age, glucose in *db/db* mice was already abnormally high at 14.5 ± 3.8 mmol/L, increasing rapidly to 24.1 ± 2.9 and 27.7 ± 6.2 mmol/L, respectively, at 7 and 8 weeks of age. At 14 and 15 weeks, they were severely hyperglycemic, with glucose levels of 30.3 ± 1.4 and 31.6 ± 0.6 mmol/L, respectively. Blood glucose in +/? mice remained normal between 6.4 ± 1.0 (14 weeks) and 7.2 ± 1.1 mmol/L (8 weeks) throughout the same time span. Therefore, even at an early age, *db/db* mice have clinical diabetes mellitus as manifested by significant hyperglycemia.

We measured the blood immunoreactive insulin levels of these 2 groups of animals (Fig 1C). Levels in *db/db* mice were 3 to 4 times the levels in +/? mice at 6 and 7 weeks of age. They remained at about the same level (759 ± 84 pmol/L) at 14 weeks, but started to decline to 386 pmol/L at 15 weeks of age. In contrast, +/? mice had blood insulin levels from 203 ± 126 pmol/L (6 weeks) to 57.6 ± 0.6 pmol/L (14 weeks).

Plasma Lipids in db/db *and +/? Mice*

We next measured plasma lipids in *db/db* and +/? mice during the same time span (Fig 1D and E). Total plasma cholesterol in *db/db* mice was consistently about 2-fold higher than the level in +/? mice (from 3.36 ± 0.60 to 4.05 ± 0.20 mmol/L in *db/db v* 1.55 ± 0.10 to 1.68 ± 0.10 mmol/L in +/?). Similarly, plasma triglycerides were about 65% higher in *db/db* animals (from 1.07 ± 0.30 to 1.25 ± 0.10 mmol/L in *db/db v* 0.66 ± 0.30 to 0.74 ± 0.10 mmol/L in +/? mice).

Plasma Lipoproteins in db/db *and +/? Mice*

We fractionated the plasma lipoproteins of *db/db* and +/? mice by FPLC.[31] This is a useful technique for separating small volumes of plasma into individual lipoprotein fractions (Fig 2). By this technique, +/? mice have essentially all of their plasma cholesterol in the form of HDL. The HDL peak is even higher in *db/db* mice. However, in these animals, we also found a very significant peak in the IDL/LDL range. By FPLC analysis, this peak is almost undetectable in the +/? plasma samples. The

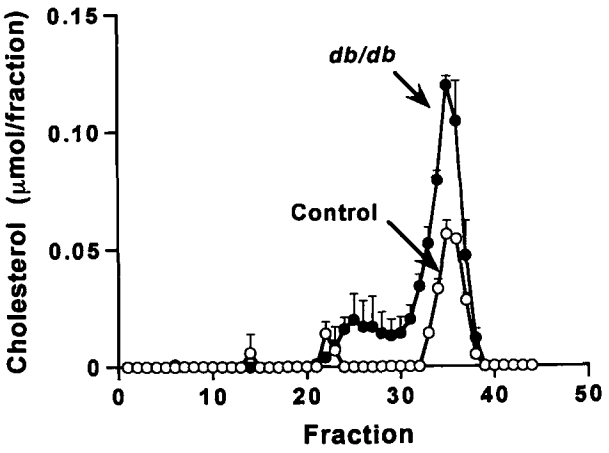

Fig 2. FPLC fraction of total plasma cholesterol in *db/db* and control (+/?) mice fed a standard chow diet. Values are the mean ± SD of 3 individual mice. The peak at fractions 20-30 represents mainly IDL/LDL, and the second peak (fractions 31-40) is mainly HDL.

distribution of plasma cholesterol in the various lipoprotein fractions was also examined by phosphotungstic acid precipitation of HDL. By this method, HDL cholesterol is 81% higher in *db/db* mice (1.48 ± 0.20 mmol/L) compared with +/? mice (0.82 ± 0.10 mmol/L, $P < .0005$). Furthermore, non-HDL (ie, VLDL/LDL) cholesterol was also higher in *db/db* versus +/? mice (1.16 ± 0.10 *v* 0.79 ± 0.20 mmol/L). This represents a 47% higher value in *db/db* versus +/? animals ($P < .01$).

Apolipoprotein Expression of db/db *and +/? Mice*

Using a previously validated standard assay for plasma apolipoproteins in *db/db* and +/? mice,[33] we found that plasma apoB-100 tended to be lower in *db/db* versus +/? mice (3.0 ± 1.0 mg/dL in *db/db v* 6.6 ± 4.0 mg/dL in +/?); however, the difference was not significant. Plasma apoB-48 in both types of mice was clearly detectable, but the concentration was too low to be accurately quantified. Plasma immunoreactive apoE concentrations were similar in both groups (3.3 ± 0.6 mg/dL in *db/db v* 3.0 ± 1.0 mg/dL in +/?), as were plasma apoA-I concentrations (130.3 ± 28.4 mg/dL in *db/db v* 161.7 ± 16 mg/dL in +/?).

To explore apolipoprotein expression at the mRNA level, we quantified the total apoB mRNA by RNase protection assay and the apoE mRNA level by Northern blot analysis of mouse liver RNA (n = 5 for +/? and 9 for *db/db*). As a control, we used β-actin, because we found that β-actin mRNA expression was comparable between *db/db* and +/? mice, whereas glyceraldehyde-3-phosphate dehydrogenase mRNA was about 50% higher in *db/db* mice (data not shown). By this analysis, *db/db* animals had similar apoB and apoE mRNA levels compared with +/? animals (mean ± SD for apoB, 100% ± 13.2% for +/? *v* 112.9% ± 20.1% for *db/db;* apoE, 100% ± 29.7% for +/? *v* 90.4% ± 40.7% for *db/db*). In humans, the liver produces almost exclusively apoB-100. In mice, the liver produces both apoB-100 and apoB-48 because of active apoB mRNA editing in the liver.[39] To determine whether the diabetic mice have an altered apoB-100/B-48 biosynthetic potential in the liver, we measured the relative amount of apoB-100 versus apoB-48 mRNA by a primer-extension assay[36] and found no difference in this ratio between the 2 groups of animals (edited mRNA, 59.9% ± 45% for +/? *v* 59.3% ± 30% for *db/db*). We also measured the relative concentration of apoB mRNA in the small intestine and found no significant difference in this parameter between *db/db* and control animals (100.0% ± 14.3% for +/? *v* 121.4% ± 28.6% for *db/db*). Intestinal apoE mRNA expression was extremely low, in agreement with previous studies in rats,[40] and could not be detected by Northern blot analysis in both types of animals. By the more sensitive RNase protection assay, the intestinal apoE mRNA concentration was similar in *db/db* and +/? mice (100.0% ± 34.7% in +/? *v* 112.2% ± 20.4% in *db/db*).

Expression of Lipolytic Activity in db/db *and Control Mice*

We measured postheparin lipolytic activity in the plasma of *db/db* and +/? mice. Using differential inhibition by 1 mol/L NaCl, we fractionated the activity into HTGL and LPL activities. Postheparin plasma HTGL was 72.4 ± 28 U/L in *db/db* versus 108.8 ± 78 U/L in +/?; the values were not different statistically (Fig 3A). Postheparin LPL activity, in contrast, was

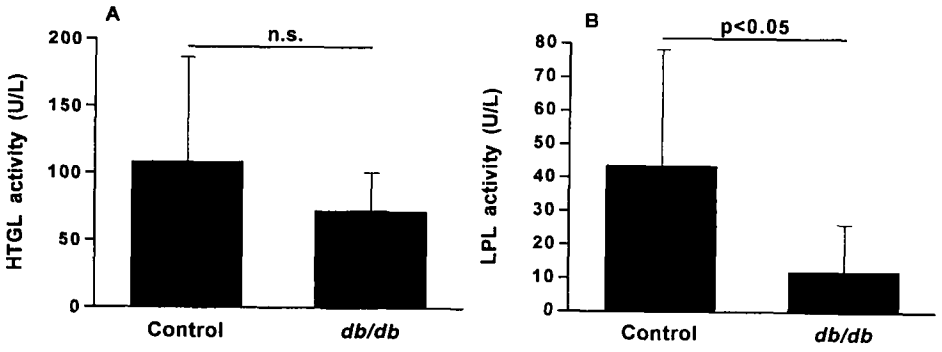

Fig 3. HTGL (A) and LPL (B) activity in *db/db* and control (+/?) mice. Postheparin lipase activities were measured after intravenous injection of heparin (100 U/kg body weight). n = 9 for both +/? and *db/db*. Values are the mean ± SD. Each measurement was performed in duplicate.

very low in *db/db* mice, being only 28% of the level in +/? mice ($P < .05$; Fig 3B).

To further examine the level of regulation of HTGL and LPL, we determined the respective mRNA levels. HTGL mRNA concentrations were similar in *db/db* and +/? mice (Fig 4A). The relative LPL mRNA concentration was determined in RNA isolated from the 3 major tissues that express the enzyme: heart, muscle, and adipose tissue (Fig 4B, C, and D). The *db/db* mice had significantly lower LPL mRNA in all tissues examined. Compared with control levels, it was expressed at 47%, 22%, and 57%, respectively, in heart, muscle, and adipose tissue. Therefore, the low postheparin LPL activity is partly mediated by differences in expression at the mRNA level.

Effect of a Western Diet on Plasma Lipids and Apolipoproteins

All lipid and lipoprotein characteristics determined in the previous sections were performed when the *db/db* and +/? mice were fed a regular chow diet. We next determined the effect of a human-like Western diet[30] on plasma lipids and apolipoproteins. Plasma cholesterol increased substantially after *db/db* and +/? mice ate a Western diet for 1 to 2 weeks (Fig 5). The hyperlipidemic response was higher in *db/db* mice. At 1 and 2 weeks on the diet, plasma cholesterol increased to 8.09 ± 0.70 and 8.80 ± 1.0 mmol/L, respectively, in *db/db* mice. In contrast, the elevations in plasma cholesterol were much more modest in +/? mice (to 3.38 ± 0.6 mmol/L at 1 week and 3.51 ± 0.7 mmol/L at 2 weeks; Fig 5A). The plasma triglyceride concentration in *db/db* mice was not significantly changed by Western diet feeding; it was consistently significantly higher than the level in +/? mice before and 1 and 2 weeks after Western diet feeding (Fig 5B).

Plasma apoB-100 increased following Western diet feeding for 2 weeks from 3.0 ± 1.0 mg/dL to 7.5 ± 2.0 mg/dL ($P < .005$) in *db/db* mice and from 6.6 ± 4.0 mg/dL to 11.3 ± 3.0 mg/dL (not significant) in +/? mice. In both types of mice, plasma apoB-48 was detectable before and after the special diet but the concentration remained too low to be accurately quantified.

We next quantified plasma apoA-I and apoE responses to Western diet feeding (Fig 5C and D). Under basal conditions, plasma apoA-I and apoE concentrations were similar in *db/db* (130.3 ± 28 mg/dL for apoA-I and 3.3 ± 0.6 mg/dL for apoE)

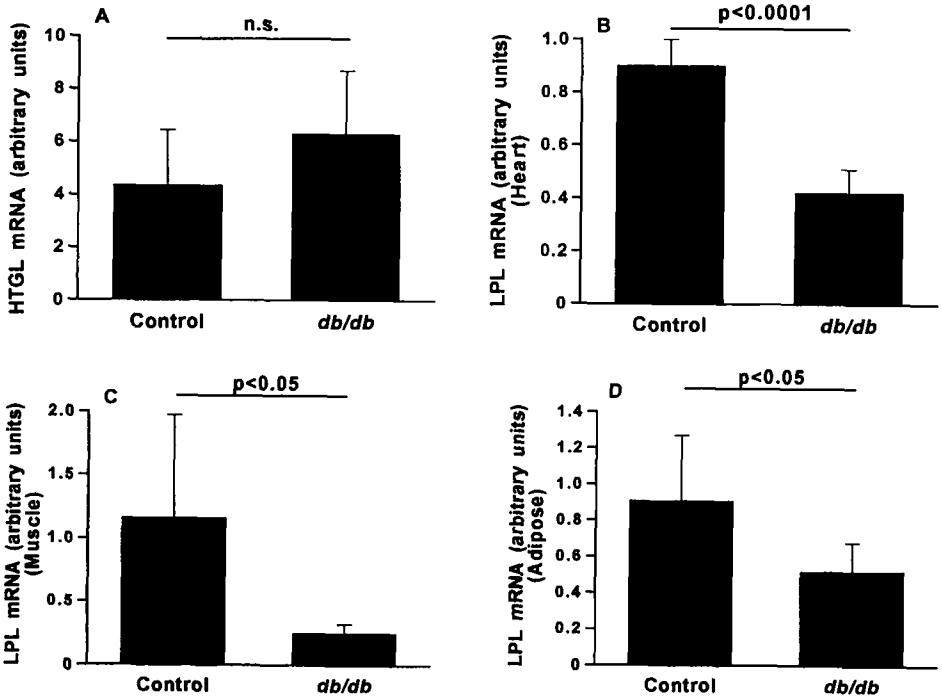

Fig 4. Relative HTGL mRNA concentration in the liver (A) and LPL mRNA expression in the heart (B), muscle (C), and adipose tissue (D) in *db/db* and control (+/?) mice. Values are the mean ± SD. RNA was isolated from the liver, heart, muscle, and adipose tissue of *db/db* and +/? mice. RNA expression levels were measured by Northern blot analysis for HTGL and RNase protection assay for LPL. They were normalized for β-actin mRNA expression. n = 5 for controls and 9 for *db/db*.

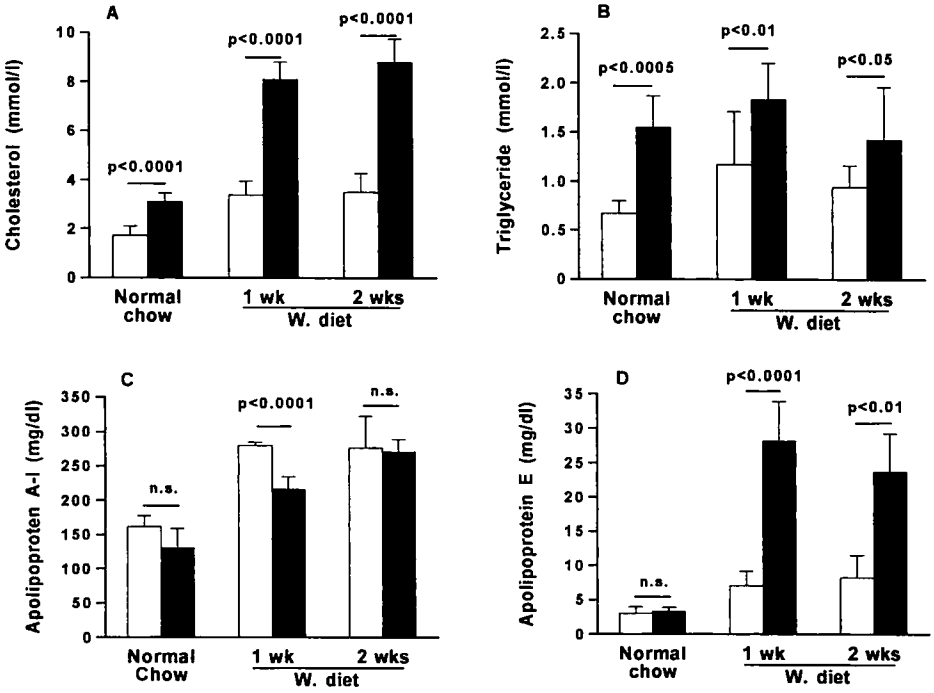

Fig 5. Effect of Western diet on plasma cholesterol (A), triglyceride (B), apoA-I (C), and apoE (D) in *db/db* (■) and control (+/?) mice (□). Values are the mean ± SD. Six-hour fasting samples were assayed enzymatically for cholesterol and triglyceride. ApoA-I and apoE were determined by radial immunodiffusion. n = 10 for all time points.

and +/? mice (161.07 ± 16 mg/dL for apoA-I and 3.0 ± 1.0 mg/dL for apoE). After Western diet feeding, apoA-I increased in both *db/db* and +/? mice. It was significantly higher in +/? mice at 1 week of diet feeding (279.0 ± 5.6 mg/dL in +/? *v* 216.4 ± 17 mg/dL in *db/db*), but after an additional week on the diet, +/? and *db/db* mice had similar plasma apoA-I concentrations (276.3 ± 45 mg/dL in +/? *v* 270.3 ± 18 mg/dL in *db/db;* Fig 5C).

Unlike apoA-I, Western diet feeding produced a much more exaggerated plasma apoE response in *db/db* versus +/? mice (Fig 5D). Within 1 week of the diet, plasma apoE increased to 28.1 ± 5.7 mg/dL in *db/db* and 7.0 ± 2.2 mg/dL in nondiabetic +/? mice. At 2 weeks, the corresponding values were 23.7 ± 5.5 mg/dL for *db/db* and 8.3 ± 3.2 mg/dL for +/? mice. The difference in plasma apoE between *db/db* and +/? was highly significant at both time points.

Effect of a Western Diet on Plasma Lipoproteins

We examined the effect of a Western diet on plasma lipoproteins in *db/db* and +/? mice. We first fractionated the plasma lipoproteins on FPLC using plasma samples from individual animals that were fed a regular chow diet and again after they were fed a Western diet for 2 weeks (Fig 6A). The diet produced a modest hyperlipoproteinemic response in +/? mice. There was a major increase in plasma HDL cholesterol and a modest increase in cholesterol under the LDL and VLDL peaks (Fig 6A). The *db/db* mice also responded with a large increase in HDL cholesterol. Most importantly, in contrast to the minimal LDL response of +/? animals, *db/db* mice responded with a massive increase in the LDL fractions (Fig 6A). The difference in the response between *db/db* and +/? animals was clearly evident when we calculated the (VLDL + LDL)/HDL ratio in the 2 types of animals at 2 weeks. Whereas +/? animals showed a 1.2-fold increase in this ratio from 0.14 to 0.17, *db/db* mice showed a 3.2-fold response, from 0.48 before diet treatment to 1.54 2 weeks following diet treatment. However, this change in the (VLDL + LDL)/HDL ratio represents an overestimation because the LDL region on the FPLC fraction overlaps with the HDL1 region.[41] The distribution of the major apolipoproteins provides additional information on the origin and properties of the various FPLC fractions. To examine the relative distribution of apoA-I, apoE, and apoB under the different lipoprotein peaks in FPLC fractionation, we performed a Western blot analysis of the FPLC fractions from mice that ate a Western diet for 2 weeks (Fig 6B). By this analysis, the apoA-I band was similar in distribution and in relative intensity in *db/db* and +/? animals. As expected, it was much more intense in the HDL fractions in both types of animals. However, small amounts of apoA-I were also evident in the "LDL" peak, indicating that small but readily detectable amounts of HDL1 contributed to this peak. As reflected by its plasma concentration, the apoE band was much more intense in *db/db* animals. This apolipoprotein was present at the highest concentration in the LDL/HDL1 fractions, but was also present in the HDL peak. The relative amount of apoB-100 and apoB-48 differed between *db/db* and +/? animals. In *db/db* animals, there was a shift in the apoB-100/B-48 ratio such that apoB-100 was the predominant species detected. It was present at a much higher concentration in the LDL/HDL1 peak and extended into the overlapping region of the HDL peak. In +/? animals, the apoB-48 band was almost as intense as the apoB-100 band and both apoB-100 and apoB-48 bands were eluted predominantly in the LDL/HDL1 region.

Effect of a Western Diet on LDL and HDL Particle Size Distribution in Control and db/db *Mice*

We isolated plasma HDL and LDL from *db/db* and +/? mice by ultracentrifugal flotation. The isolated HDL and LDL were

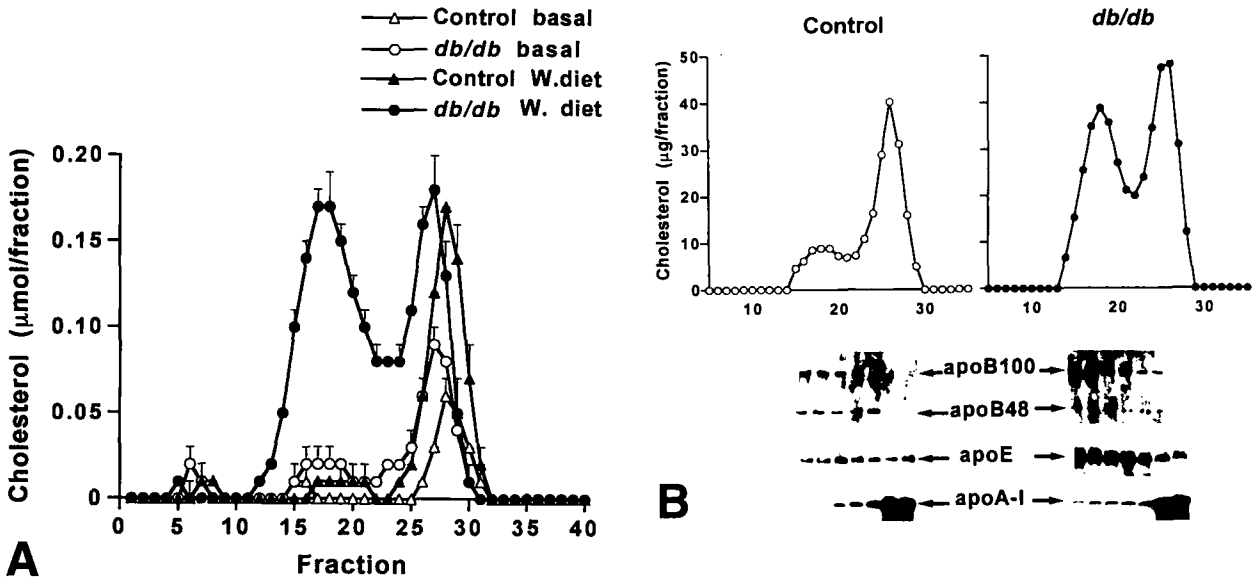

Fig 6. Effect of Western diet on FPLC fractionation of total plasma cholesterol (A) and Western blot analysis of lipoprotein fractions (B). (A) Values are the mean ± SD of 3 individual animals. The first peak at fractions 4-10 represents VLDL, the second peak (fractions 12-22) IDL/LDL, and the third peak (fractions 23-33) HDL. (B) FPLC analyses were repeated with plasma samples pooled from 3 control (+/?) or 3 *db/db* mice that were fed a Western diet for 2 weeks. Western blots were performed on the fractions against apoB, apoE, and apoA-I antibodies.

examined with nondenaturing gradient gels to ascertain whether there were major shifts in particle distribution between +/? and *db/db* mice and whether the Western diet influenced lipoprotein size and mass distribution. Figure 7 shows that HDLs from +/? mice on a chow diet have a symmetrical peak at 9.7 nm, while in chow-fed *db/db* mice the HDL peak is shifted to somewhat larger, less dense particles, consistent with the observation that HDL cholesterol (Fig 2) is increased in these mice compared with +/? mice even though their apoA-I concentrations are similar. A Western diet results in only a very modest change in HDL size for either the +/? or *db/db* mouse. The most striking difference between control and diabetic mouse lipoproteins is noted in the LDL distribution profile (Fig 7). The +/? mice have a major LDL peak at 26.6 nm and a broad shoulder at 20.7 nm similar to that previously reported for chow-fed mice[42]; this profile does not change with fat feeding. Chow-fed *db/db* mice have a major peak of small dense LDL (21.4 nm) and a shoulder at 17.5 nm. During fat feeding, small dense LDLs persist but additional larger particles with a diameter of 26 to 27 nm also appear (Fig 7); the latter could account for the large increase in LDL cholesterol noted on FPLC (Fig 6A) of *db/db* mouse plasma. Particles in the small-pore region of the gel (16 to 20 nm) are likely to represent HDL1 particles, since both apoA-I and apoE were noted in FPLC fractions of LDL (Fig 6B).

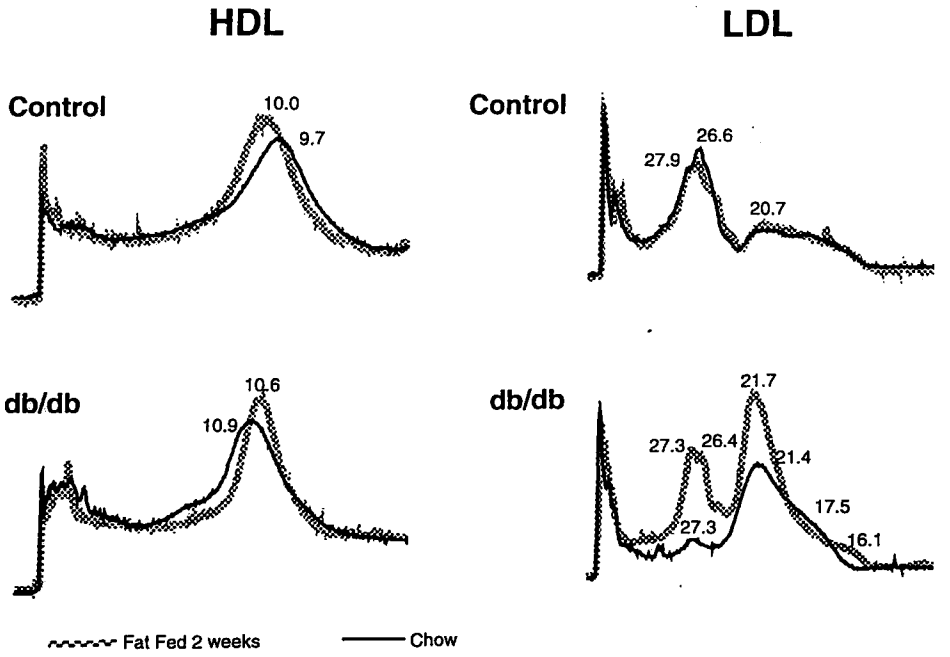

Fig 7. Nondenaturing gradient polyacrylamide gel profiles of HDL and LDL from control (+/?) and *db/db* mice. LDL and HDL were first isolated by KBr ultracentrifugation and then electrophoresed on 2%-16% and 4%-30% gels, respectively. The numbers at the peaks represent particle diameter in nm.

DISCUSSION

The diabetes (*db/db*) mouse has been used as an animal model of type 2 diabetes for over 30 years.[43] It was only in 1996 that the specific mutation in the diabetes gene was fully characterized; it was found to be a G → T point mutation that resulted in the production of an abnormally spliced mRNA for the leptin receptor.[26] This mutation leads to defective signal transduction.[44] Leptin receptor missense mutations were also found in fatty Zucker[27] and corpulent[28] rats. The *db/db* mouse develops obesity, insulin resistance, hyperglycemia, and resistance to leptin. As early as 6 weeks after birth, *db/db* mice are already markedly obese with substantial fasting hyperglycemia, which continues to worsen throughout life. There is also marked hyperinsulinemia (Fig 1C), indicating that *db/db* mice do not have absolute insulin deficiency but instead display insulin resistance. Furthermore, as observed by Sinha et al,[45] at 15 weeks, there is a decrease in plasma insulin, suggesting β-cell failure. Thus, the *db/db* mouse is a model for severe type 2 diabetes associated with obesity. As noted by Nishina et al,[29] these animals also have basal hypercholesterolemia and hypertriglyceridemia while fed regular laboratory chow (Fig 1). Like wild-type mice in general, *db/db* mice are not prone to develop atherosclerosis. However, a careful analysis of their lipoprotein abnormalities may provide insight into the dyslipidemia associated with type 2 diabetes.

To further define the lipid abnormalities in diabetic *db/db* mice, we compared the lipoprotein profile (as analyzed initially on FPLC) of total plasma in *db/db* and their genetically matched nondiabetic controls (+/?). It is clear that the hypercholesterolemia was a result of elevations in both LDL and HDL (Fig 2). These lipoprotein abnormalities were not associated with any significant changes in plasma apoB, apoA-I, or apoE concentrations or hepatic or small-intestinal apoB or apoE mRNA levels. The abnormality of the lipoprotein profile in *db/db* mice is different from that in Zucker or corpulent rats, although they all have mutations in the leptin receptor. The rats exhibit a marked increase in triglyceride and a moderate increase in cholesterol, which are evident mostly in the VLDL and HDL fractions.[46-48]

A deficiency in postheparin lipolytic activity has been well documented in patients with type 2 diabetes.[49] Insulin resistance appears to be 1 factor associated with low postheparin LPL activity.[50] In *db/db* mice, there is marked LPL deficiency as reflected by the greatly reduced postheparin LPL activity. Tissue LPL mRNA levels are also moderately to markedly reduced, indicating that part of the LPL deficiency is a result of low LPL mRNA expression. It is likely that other defects also contribute to the markedly reduced LPL activity in the insulin-resistant *db/db* mice, because insulin deficiency has been shown to adversely affect LPL function in 3T3-L1 adipocytes at a posttranslational level.[51] This lower LPL activity may have caused a modest increase in VLDL cholesterol by FPLC analysis (Fig 6) and in the relative apoB-100/B-48 ratio. Such defective removal of VLDL has been reported in *db/db* mice.[52] The reduction in HTGL activity has been reported in patients with type 2 diabetes.[53-55] However, we did not find a significant reduction in HTGL activity in *db/db* mice compared with +/? mice. These results suggest that the primary cause of the increase in LDL cholesterol in *db/db* mice may be a defective removal of these lipoproteins. In contrast, adipose tissue LPL activity was reported to be increased[56] and postheparin HTGL activity was elevated[57] in Zucker rats. Hyperlipidemia in these rats is caused mainly by hypersecretion of VLDL and possible saturation of the lipolytic mechanism responsible for the removal of triglyceride-rich lipoproteins.[57]

In comparison to +/? mice, *db/db* mice display distinctive dyslipidemia. However, when the mice are fed regular laboratory chow, the abnormalities tend to be relatively mild (Fig 2). The picture is quite different when *db/db* mice are challenged with a Western diet (Fig 6). Unlike their genetically matched nondiabetic controls, *db/db* mice overrespond to this diet with marked elevations in plasma cholesterol and, to a lesser extent, plasma triglyceride. The calculated (VLDL + LDL)/HDL cholesterol ratio as determined by FPLC in *db/db* mice increases about 3-fold, predominantly due to an increase in the LDL fraction. However, we note that the calculated ratio represents an overestimate because some HDL1 overlaps LDL in this region, as shown by the presence of small amounts of apoA-I in these fractions. Nevertheless, the true (VLDL + LDL)/HDL ratio must be significantly higher in *db/db* mice compared with their nondiabetic controls fed the same diet. Thus, the Western diet–fed *db/db* mice are a better model for type 2 diabetes than the animals fed regular chow, since their dyslipidemia is more similar to that in diabetic humans. One interesting characteristic of LDL isolated from *db/db* mice, whether fed regular chow or a Western diet, is the predominance of small dense LDL (Fig 7).

In humans, individuals with a predominance of small dense LDL (phenotype pattern B) have an increased risk for coronary heart disease.[53] This phenotype is associated with increased plasma triglyceride, insulin, and glucose and decreased HDL.[54] Small dense LDL is also one of the characteristics of diabetic dyslipidemia.[5,6,10-16,19] It has been demonstrated in a group of elderly men and women that small dense LDL is a risk factor in non–insulin-dependent diabetes.[55] The *db/db* mouse model has small dense LDL and, in addition to this atherogenic LDL profile, it has many of the other characteristics of type 2 diabetes including impaired postheparin LPL activity and elevated triglyceride, glucose, and insulin,[3,4,29] which suggests that it may be a useful model for studying the metabolism of apoB-containing lipoproteins in diabetes. Interestingly, in the *db/db* mouse, diabetes is not associated with a diminution of HDL, which tends to increase in mass in this model. This increase may be associated in part with the lack of cholesteryl ester transfer protein[58] in these mice, which leads to an accumulation of large HDL.

In summary, the *db/db* mouse appears to be a useful model for the dyslipidemia associated with type 2 diabetes, especially when it is fed a Western diet. We have examined the molecular pathology of its dyslipidemia and present a detailed characterization of the lipoprotein abnormality. This model should be well suited for future studies on the pathogenesis and treatment[20,59,60] of dyslipidemia associated with type 2 diabetes.

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