Insulin-Treated Zucker Diabetic Fatty Rats Retain the Hypertriglyceridemia Associated With Obesity

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Lipoprotein and apolipoprotein changes were evaluated in 10-week-old Zucker diabetic fatty (ZDF) male rats following 12 weeks of insulin treatment, which normalized blood glucose and maintained weight gaining characteristic of nondiabetic Zucker fatty rats. Compared with untreated ZDF rats (saline-injected), insulin treatment resulted in increased very–low-density lipoprotein (VLDL; $d < 1.006$ g/mL) and decreased alpha lipoprotein on agarose gel electrophoresis. These findings were consistent with an observed increase in VLDL triglyceride and cholesterol, and decreased high-density lipoprotein (HDL) cholesterol with insulin treatment in isolated lipoproteins. B100 levels were unchanged by insulin treatment, but B48 levels were significantly increased in the VLDL fraction. Insulin treatment depressed apolipoprotein (apo) A-I levels in HDL, but had little effect on total apo E, apo A-IV, or apo C, although apo C was redistributed to the VLDL fraction. These results suggest that insulin treatment of ZDF rats normalizes hyperglycemia and prevents age-related changes in lipoprotein parameters associated with development of insulinopenic diabetes. Insulin therapy in ZDF rats thereby sustains the hyperlipidemic lipoprotein pattern associated with hyperinsulinemia and obesity.

THE EFFECT OF long-term insulin therapy on lipoprotein parameters in type 2 diabetes has not been well studied.[1] One reason is the uncertainty associated with the potentially negative impact of insulin treatment on lipoprotein metabolism and atherogenesis. Recently, the male Zucker diabetic fatty rat (ZDF/Gmi [Genetic Models, Indianapolis, IN] *fa/fa*) has been introduced as a model for human type 2 diabetes.[2,3] The ZDF rat is derived from the Zucker obese rat, but differs from the parent strain in that ZDF rats consistently develop hyperglycemia. This is believed to be a result of a second diabetogenic mutation, distinct from the *fa* gene, possibly related to an intrinsic pancreatic β-cell defect.[4] Progression of diabetes in ZDF rats parallels the natural history of human type 2 diabetes. At about 9 to 10 weeks of age, ZDF rats develop hyperinsulinemic hyperglycemia and at about 20 weeks, they develop insulinopenic hyperglycemia related to β-cell exhaustion.

The current study was undertaken to determine the impact of long-term insulin treatment of male ZDF rats on serum lipid, lipoproteins, and apolipoproteins. The question addressed was whether the hyperinsulinemic Zucker and ZDF lipoprotein pattern would be retained when insulinopenia was prevented by insulin treatment. Results suggest that insulin treatment of ZDF rats appears to compensate for the pancreatic β-cell defect and normalizes blood glucose, but fails to correct the hyperlidemia associated with obesity. Lipoprotein and apolipoprotein patterns obtained support the finding that insulin-treated ZDF rats retain the hyperlipidemic phenotype associated with the obese state.

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METHODS

Animals

Male, ZDF (ZDF/Gmi, *fa/fa*) were from Genetic Models. Ultra Lente insulin (U-100) was obtained from Lilly (Indianapolis, IN). At 10 weeks of age, insulin was administered subcutaneously as a single dose at 3 PM (initial dosage, 27 U/kg).[5] Plasma glucose and body weights were determined on Tuesday and Friday mornings. Insulin doses over the 12 weeks of therapy were adjusted to maintain plasma glucose at or below 200 mg/dL. Rats had ad libitum access to Purina Rodent Chow 5008 (Richmond, IN) and were studied in the fed state. Animal protocols were approved by the University Committee on Animal Resources of the University of Rochester.

Biochemical Methods

Serum glucose levels were measured by an automated hexokinase and glucose-6-phosphate dehydrogenase assay[6] on a Synchron CX system (Beckman Instruments, Fullerton, CA). Serum cholesterol levels were measured using a cholesterol oxidase method[7] and serum triglycerides by assay of glycerol released following lipase treatment.[8] Free fatty acids were determined using the NEFA-C kit (Wako, Biochemical Diagnostics, Edgewood, NY). Serum lipoprotein electrophoresis was performed on agarose gels using the Universal Gel/8 system (Ciba-Corning Diagnostics, Palo Alto, CA).

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

Lipoproteins were isolated by sequential density ultracentrifugation of serum[9] after addition of EDTA (final concentration, 2.5 mmol/L). Very–low-density lipoprotein (VLDL) was isolated from serum ($d < 1.006$ g/mL) and then the salt density was adjusted to 1.063 g/mL for low-density lipoprotein (LDL) isolation and 1.210 g/mL for high-density lipoprotein (HDL) isolation by addition of solid NaBr. Lipoproteins were isolated by flotation at 14°C at 50,000 rpm in an 80-Ti rotor for 14 to 16 hours for VLDL, 22 hours for LDL, and 40 hours for HDL. Isolated lipoproteins were dialyzed against a total of 6 L of 0.15-mol/L NaCl/2.5 mmol/L EDTA at 4°C using a 3,500–molecular weight cut-off dialysis membrane with three to four changes of dialysate. The protein content of each fraction was then assayed[10] to determine total apolipoprotein in each lipoprotein fraction. To delipidate lipoproteins, 1 mL of dialyzed lipoprotein was added to 20 mL chloroform/methanol/diethyl ether, 5:5:10, vol/vol/vol, and the mixture

was stored at −20°C in an explosion-proof freezer. For analysis, apolipoproteins were pelleted by slow-speed centrifugation and air-dried after rinsing the pellet with diethyl ether. Pellets were dissolved in sample buffer[11] containing freshly added dithiothreitol (final concentration, 10 mmol/L) and heated to 95 to 100°C for 5 to 10 minutes before applying to gels. Apolipoproteins were separated on linear gradient gels cast onto GelBond (FMC BioProducts, Rockland, ME).[12,13] Gradients were 3.5% wt/vol to 26% wt/vol acrylamide/Acrylaide gels for VLDL and LDL apolipoproteins, and 10.5% wt/vol to 26% wt/vol acrylamide/Acrylaide (FMC BioProducts) gels for HDL apolipoproteins. Gels were stained and individual apolipoproteins identified and quantitated by scanning densitometry as previously described.[14] The apolipoprotein (apo) C portion of the gels includes the low–molecular weight apolipoproteins, apo C-III, apo C-II, and apo A-II. Since apolipoproteins may vary with respect to dye binding,[15] the results reported are estimated levels of the mass of apolipoprotein.

Gel Filtration

Ultracentrifuged VLDL ($d < 1.006$ g/mL) isolated from pooled sera derived from saline-injected or insulin-treated ZDF rats were size-fractionated by gel filtration column chromatography on 1.5- × 100-cm columns (Econo-Columns; Biorad, Hercules, CA). Columns were packed with 4B-CL Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) in 0.01 mol/L phosphate, pH 7.4, containing 0.15 mol/L NaCl, and lipoproteins were eluted using the same buffer at a rate of 5 mL/h with fractions collected every 30 minutes. Fractions were then analyzed for triglyceride[8] and for apo B.[16]

Statistical Analysis

Results are expressed as the mean ± SD unless indicated otherwise. Statistical comparisons were performed using Student's *t* test for unpaired data.

RESULTS

Daily subcutaneous insulin injection of male ZDF rats was effective in lowering glucose levels from an average of 450 to 500 mg/dL to target levels ≤200 mg/dL (Fig 1A). During this time course, the weight gain of untreated diabetic rats was significantly less than that of insulin-treated ZDF rats, averaging 7.5 g/wk compared with 33.4 g/wk, respectively (Fig 1B). By the end of the study (70 days), insulin-treated ZDF rats weighed an average of 690 ± 12 g, similar to that expected for nondiabetic Zucker obese rats of similar ages (672 ± 22 g).[14] The weight of untreated diabetic ZDF rats averaged 424 ± 8 g, similar to the average weight of age-matched lean ZDF rats (416 ± 31). Serum cholesterol levels were 25% lower ($P < 0.037$) in insulin-treated ZDF rats compared with untreated controls (mean ± SD): 212 ± 39 mg/dL (n = 6) versus 266 ± 19 mg/dL (n = 4). In contrast, serum triglyceride levels were on average 28% higher in insulin-treated compared with untreated ZDF rats (mean ± SD): 1,328 ± 439 mg/dL (n = 6) versus 950 ± 115 mg/dL (n = 4). Serum free fatty acids were comparable in treated and untreated ZDF rats (mean ± SD): 2.37 ± 0.51 mEq/L (n = 6) versus 2.56 ± 0.31 mEq/L (n = 4).

To examine the effect of insulin therapy on lipoproteins, agarose gel electrophoresis was performed on normal sera (Fig 2A) and on sera where the VLDL fraction was removed by ultracentrifugation (Fig 2B). The serum lipoprotein pattern of untreated ZDF rats demonstrated significant staining of pre–beta-, beta-, and alpha-migrating lipoprotein bands. The insulin-treated ZDF rat showed broader staining extending from the point of application to the pre-beta area. In untreated ZDF rats,

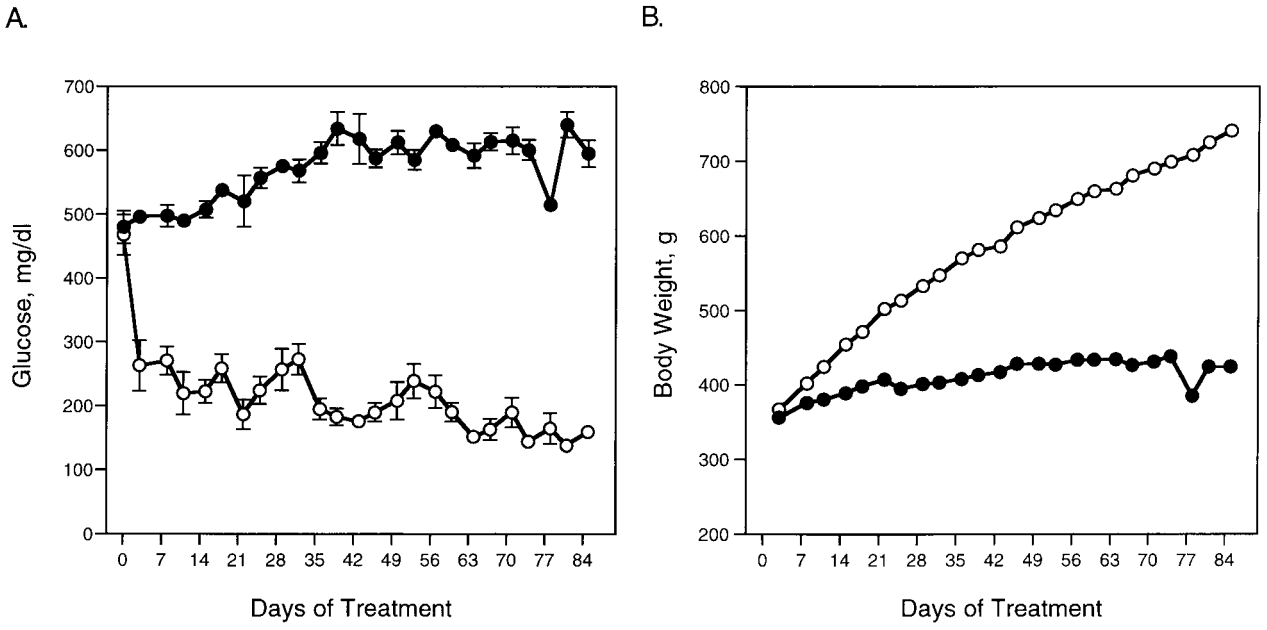

Fig 1. Effect of insulin treatment on serum glucose levels (A) and weight gain of Zucker diabetic rats. Ten-week-old ZDF rats were treated by injection with saline (●—●) or with Ultra Lente U-100 insulin (○—○) subcutaneously for 12 weeks. Blood glucose levels and body weights were determined on Tuesday and Friday of each week in the morning. (A) Results are the mean glucose levels ± SD (N = 10 rats each condition at each time point). (B) Results are average body weights of 10 rats in each group at each time point ± SEM. Error bars are too small to be visible, but averaged less than 2.3% of the mean body weight over the course of the treatment period. The average insulin dosage (U/kg ± SD) given ZDF rats subcutaneously over the 12-week period in three 4-week intervals was 45.3 ± 10.1, 89.5 ± 6.7, and 101.9 ± 2.7.

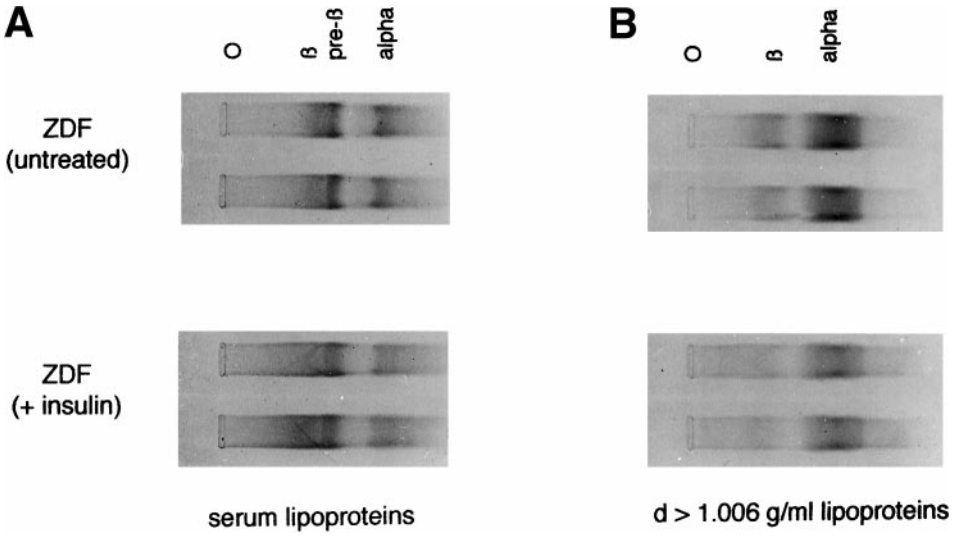

Fig 2. Serum lipoprotein electrophoresis on agarose gels. Lipoprotein electrophoresis was performed on serum (A) and on serum in which VLDL ($d < 1.006$ g/mL) were removed by ultracentrifugation flotation (B). Results are from 2 representative ZDF rats injected with saline and 2 insulin-treated ZDF rats.

the predominant lipoprotein removed by ultracentrifugation had pre-beta mobility consistent with VLDL. In insulin-treated ZDF rats, staining disappeared from the point of application to the pre-beta region, leaving less discrete staining. This is consistent with a VLDL fraction present in insulin-treated ZDF rats with an unusual composition.

To better evaluate the effect of insulin treatment on specific lipoprotein fractions, VLDL ($d < 1.006$ g/mL), LDL (1.006 g/mL $< d <$ 1.063 g/mL), and HDL (1.063 g/mL $< d <$ 1.210 g/mL) were isolated by sequential density ultracentrifugation. Isolated lipoproteins were dialyzed and cholesterol and triglyceride content (Table 1), and total apolipoprotein content were determined (Table 2). Compared with untreated rats, insulin treatment of ZDF rats increased VLDL triglyceride by 38% and cholesterol by 28% while decreasing HDL cholesterol by 38%. Changes in VLDL lipids in insulin-treated ZDF rats paralleled a 38% increase in VLDL total apolipoprotein concentration. The decrease in HDL cholesterol in insulin-injected rats corresponded to a 27% decrease in HDL total apolipoprotein concentration. LDL total apolipoprotein concentration was marginally decreased with insulin treatment and there was a small but significant decrease of LDL cholesterol of approximately 20%.

To further characterize changes in VLDL that occurred with insulin treatment, gel filtration column chromatography was used to size-fractionate VLDL ($d < 1.006$ g/mL) isolated by ultracentrifugation of pooled sera derived from untreated and insulin-treated ZDF rats (Fig 3). Triglyceride (Fig 3A) and apo B (Fig 3B) concentrations of column fractions were plotted against elution volume. As apo B measures the number of lipoprotein particles present,[17] results suggest that insulin treatment led to an increased proportion of very large VLDL at the expense of smaller particles in the very low density fraction ($d < 1.006$ g/mL).

To obtain additional information regarding apolipoprotein changes in lipoprotein fractions following insulin treatment, B100 and B48, as well as apo E, apo C, apo A-I, and apo A-IV, were analyzed by sodium dodecyl sulfate–polyacrylamide gel electropheresis (SDS-PAGE) and scanning densitometry for estimation of apolipoprotein concentrations as previously described.[14] The results for B100 and B48 (Fig 4) and the ratio of B48 to B100 mass in VLDL and LDL (Table 3) are summarized separately. Insulin treatment of ZDF rats significantly increased B48 in VLDL and decreased B100 in the LDL fraction. These changes are consistent with an altered mass ratio of B48 to B100 in VLDL, LDL, and VLDL + LDL that increased with insulin treatment compared with fractions derived from untreated ZDF rats.

Because the molar concentration of apo B yields an estimate of the number of lipoprotein particles,[17] we calculated molar concentrations of B100 and B48 in VLDL and LDL using reported molecular weights of 500 and 250 kd, respectively.[18] The total number of serum apo B-containing lipoproteins (VLDL + LDL) was somewhat greater in insulin-treated compared with untreated rats, although their mean values were not significantly different: 723 ± 106 nmol/L (n = 6) versus 591 ± 87 nmol/L (n = 4). However, the distribution of B100 and B48 within VLDL and LDL fractions differed substantially. VLDL of untreated and insulin-treated ZDF rat sera contained equivalent numbers of B100-VLDL (mean ± SD): 149 ± 22 nmol/L

Table 1. Lipid Composition of Serum Lipoprotein Fractions Derived From Saline-Injected and Insulin-Treated Male ZDF Rats

		VLDL*		LDL*	HDL*
Group	N	Triglyceride	Cholesterol	Cholesterol	Cholesterol
Saline	4	909 ± 128	49 ± 8	34 ± 3	117 ± 21
Insulin	6	1,255 ± 82†	63 ± 9‡	27 ± 3‡	73 ± 7†

*Values (mg lipid per dL serum) are expressed as the mean ± SD.

†$P < .001$.

‡$P < .05$.

Table 2. Apolipoprotein Content of Isolated Lipoprotein Fractions Derived From Saline-Injected and Insulin-Treated Male ZDF Rats

Group	N	VLDL*	LDL*	HDL*
Saline	4	86.7 ± 8.4	35.0 ± 5.1	238.8 ± 8.7
Insulin	6	119.4 ± 9.6†	29.5 ± 7.8	175.2 ± 12.7†

*Values (mg protein per dL of serum) are expressed as the mean ± SD.

†$P < .001$.

(n = 4) versus 159 ± 43 nmol/L (n = 6). However, the number of B48-containing VLDL was increased significantly ($P < .001$) following insulin therapy (mean ± SD): 511 ± 60 nmol/L (n = 6) versus 312 ± 56 nmol/L (n = 4). There were also more B100-LDL particles in untreated ZDF rats ($P < .001$) than in insulin-treated ZDF rats (mean ± SD): 74 ± 10 nmol/L (n = 4) versus 21 ± 12 nmol/L (n = 6). The number of B48-LDL was much more variable in untreated and insulin-treated ZDF rats (mean ± SD): 56 ± 22 nmol/L (n = 4) versus 32 ± 14 nmol/L (n = 6).

With regards to the effect of insulin treatment on non-apo B apolipoproteins, only apo A-I concentration was affected by insulin treatment (Fig 5), demonstrating a 33% decrease compared with untreated ZDF rats ($P < .001$). The decrease in serum apo A-I was due to decreased apo A-I in HDL, a finding consistent with the observed decrease in HDL cholesterol and total HDL apolipoprotein. Compared with untreated ZDF rats, insulin treatment led to a shift of apo E from LDL to HDL and a shift of apo C from HDL to the VLDL fraction.

DISCUSSION

The current study assessed the effect of insulin treatment of diabetes on serum lipoproteins in ZDF rats. Previous studies from our laboratory suggest that two lipoprotein patterns can be defined according to relative insulin levels.[14] The first is a pattern associated with hyperinsulinemic diabetes that occurs between 9 and 10 weeks of age. The second is a pattern associated with insulinopenic diabetes that occurs around 20 weeks of age. In the current study, beginning at 10 weeks of age, diabetic ZDF rats were treated either with saline or insulin for 12 weeks. During this time period, hyperinsulinemia progresses to insulinopenia when animals are not treated. At the end of the experiment, serum lipids and lipoproteins of insulin-treated diabetic rats were compared with those of untreated ZDF rats (saline-injected). Overall, the lipoprotein pattern of insulin-treated ZDF rats resembled that of the hyperinsulinemic ZDF rat and of the nondiabetic Zucker obese rat, demonstrating sustained increases in VLDL lipid, B48, and apolipoprotein components associated with accentuated hypertriglyceridemia. Insulin treatment of ZDF rats also led to more rapid weight gain than in untreated ZDF rats, which was comparable to nondiabetic Zucker obese rats.

As anticipated, by 22 weeks of age, untreated ZDF rats developed the pattern associated with insulinopenic diabetes that included the enrichment of LDL and HDL with cholesterol and an increase in apolipoproteins in these lipoprotein fractions.[14] Consistent with these changes were electrophoretic patterns demonstrating increases in beta- and alpha-migrating lipoproteins. Treatment of ZDF rats with insulin resulted in decreased cholesterol and apolipoprotein content of isolated LDL and HDL and a corresponding decrease in staining of beta- and alpha-migrating lipoproteins on electrophoresis. Insulin-treated ZDF rats had higher VLDL lipids (triglyceride and cholesterol) and apolipoproteins with a broad staining pattern extending to the point of application consistent with VLDL,

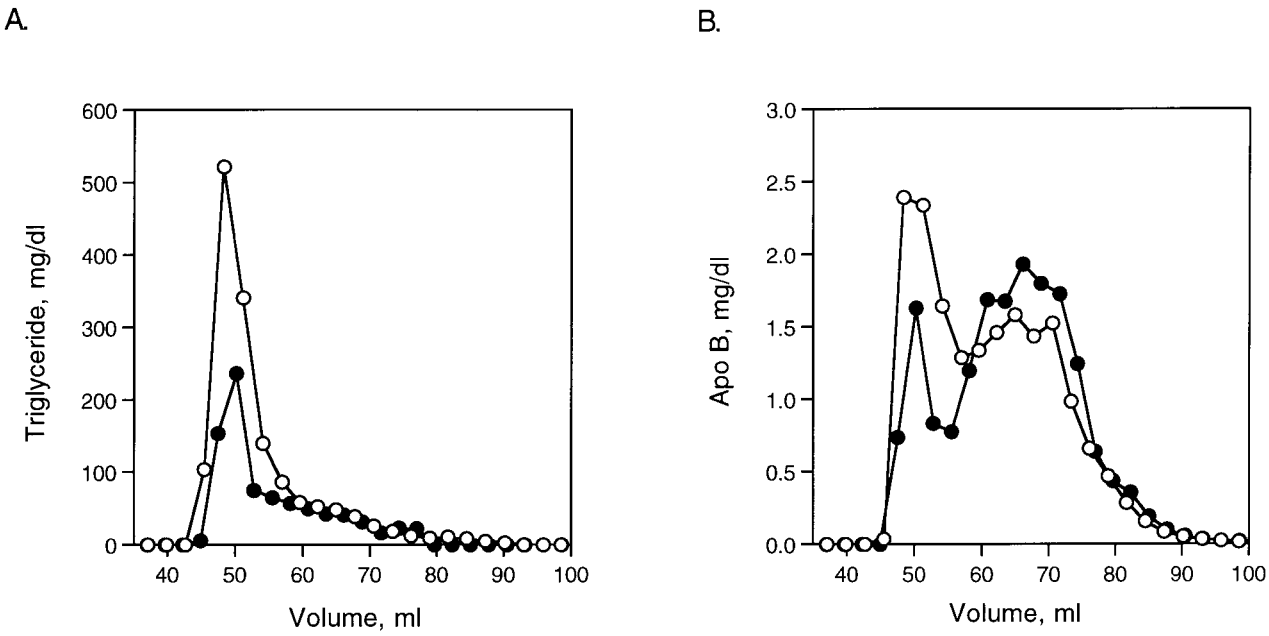

Fig 3. Gel filtration column chromatography of $d < 1.006$ g/mL lipoproteins derived from saline-injected and insulin-treated ZDF rats. Triglyceride-rich lipoproteins with densities less than 1.006 g/mL were isolated from a serum pool derived from 4 saline-injected (●—●) or 6 insulin-treated (○—○) ZDF rats at the end of the 12-week treatment period. Lipoproteins were separated by gel filtration on columns of 4B-CL Sepharose and were eluted in phosphate-buffered saline. After separation, each column fraction was analyzed for triglyceride (A) and apo B (B); results are plotted against elution volume.

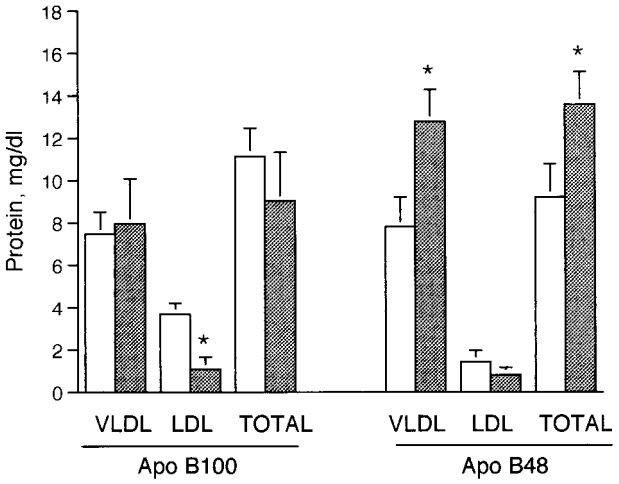

Fig 4. Distribution of apo B100 and B48 in VLDL and LDL fractions isolated by ultracentrifugation. VLDL and LDL derived from serum of saline-injected (□) and insulin-treated ZDF rats (■) were isolated by ultracentrifugation. After delipidation, apolipoproteins were separated by SDS-PAGE, stained with Coomassie blue, and B100 and B48 concentrations estimated by densitometry as described in the Methods. Results are the mean ± SD from 4 saline-injected and 6 insulin-treated ZDF rats.

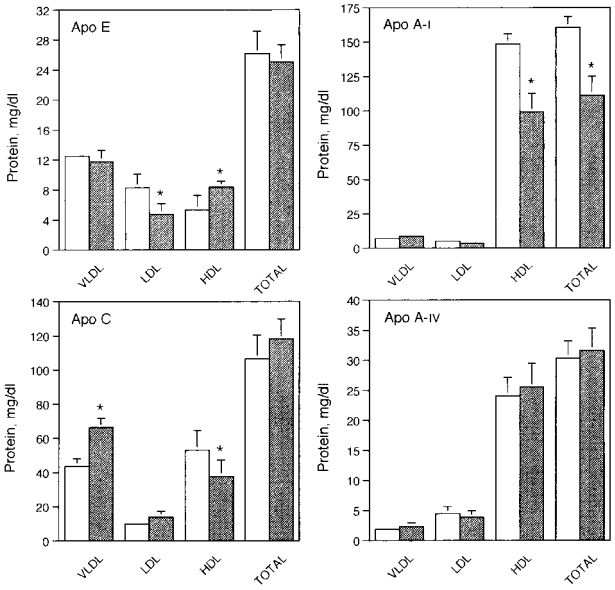

Fig 5. Serum apo E, apo A-I, apo C, and apo A-IV concentrations (mg protein/dL) in VLDL, LDL, HDL, and in total lipoproteins of saline-injected (□) and insulin-treated (■) ZDF rats following therapy. VLDL, LDL, and HDL were isolated by sequential density ultracentrifugation, dialyzed to remove salts, and delipidated. Delipidated apolipoproteins in each fraction were solubilized in SDS, separated by SDS-PAGE, and gels stained. Stained gels were analyzed by densitometry to obtain an estimate of apolipoprotein concentrations. Results are from 4 saline-treated and 6 insulin-treated rats and are expressed as the mean ± SD.

chylomicrons and their respective remnants. This staining pattern was associated with larger, triglyceride-rich particles as shown by gel filtration.

In the current study, insulin treatment of ZDF rats increased significantly the mass ratio of B48 to B100 in apo B–containing lipoproteins compared with untreated ZDF rats. The increased mass ratio in insulin-treated rats reflects increased numbers of VLDL-B48 particles and is consistent with the hyperinsulinemic pattern observed in 10-week-old ZDF and Zucker fatty rats. Decreased LDL-apo B with insulin treatment is associated with decreased B100-LDL. The increase in B48 with insulin treatment may relate to increased hepatic expression of APOBEC-1, the enzyme responsible for apo B mRNA editing. In Zucker fatty rats, hepatic apo B mRNA editing is stimulated[19] and VLDL-B48 production by liver is increased.[20] Alternatively, there could be increased contribution of intestinal lipoproteins containing B48 in insulin-treated ZDF rats.

The Zucker obese strain of rats is characterized by increases in apo C,[21] including apo C-III,[22] and enrichment of apo C in the VLDL fraction,[22] which is also observed in ZDF rats.[14] Insulin treatment of ZDF rats shifted apo C to the VLDL fraction without a comparable shift of apo E. Using apo B as a determinant of particle number, the molar ratio of apo E to apo B in VLDL decreased by one third from 8.38 ± 1.33 (n = 4) in untreated ZDF rats to 5.37 ± 0.52 (n = 6) in insulin-treated rats. The net result was to increase the ratio of apo C to apo E on the average VLDL particle. An increased ratio of apo C to apo E is a known determinant in the catabolism of VLDL,[23,24] making the particle less favorable for removal. Thus, insulin treatment leads to changes in VLDL that would be expected to intensify hypertriglyceridemia.

Table 3. Ratio of B48 to B100 Mass in Serum VLDL and LDL Derived From Saline-Injected and Insulin-Treated Male ZDF Rats

Group	N	VLDL*	LDL*	VLDL + LDL*
Saline	4	1.04 ± 0.08	0.38 ± 0.11	0.82 ± 0.06
Insulin	6	1.67 ± 0.28†	0.78 ± 0.19‡	1.56 ± 0.25†

*Results are expressed as the mean ± SD.
†$P < .002$.
‡$P < .006$.

HDL cholesterol, apo HDL, and apo A-I increase substantially in untreated ZDF rats as diabetes progresses from hyperinsulinemia to insulinopenic hyperglycemia.[14] The increase in apo A-I in 20-week ZDF rats is as much as 3 times that observed at 10 weeks of age, and similar elevations are observed in the current study of untreated ZDF rats. In insulin-treated ZDF rats, HDL and apo A-I were lower than in untreated ZDF rats, suggesting that insulin treatment suppresses the increase in HDL that would be expected in untreated 20-week ZDF rats. Recent studies suggest that insulin and glucose have opposing actions on the rat hepatic apo A-I promoter and on apo A-I expression.[25] However, when glucose and insulin are combined, promoter activity is still stimulated. Parallel changes are observed in liver derived from fructose-fed rats where hepatic apo A-I protein and mRNA levels are also increased.[25] Hence, insulin treatment and lowered blood glucose should have resulted in increased levels of hepatic apo A-I. The reason for lower HDL and apo A-I with insulin treatment is unknown, although other hormonal and metabolic changes are present in Zucker *fa/fa* rats.[14] In addition, the intestinal contribution to serum apo A-I cannot be ignored.

A difference observed previously between Zucker obese and ZDF rats is the slower rate of growth of ZDF rats attributed to

the diabetic state.[14] As observed here, insulin treatment resulted in rapid weight gain, which was significantly greater than that observed in either lean or untreated ZDF rats. Interestingly, the weight gained by insulin-treated ZDF rats was essentially equivalent to that attained by nondiabetic Zucker obese rats of similar age. Although food intake was not part of the experimental design, future studies will be necessary to address whether the rapid weight gain of ZDF rats due to insulin treatment was associated with increased food intake or increased food efficiency.

In humans with type 2 diabetes, insulin therapy produces a general improvement of the associated hyperlipidemia, including reduced triglyceride and apo B and increased HDL.[26,27] Thus, it appears that insulin treatment of the diabetic ZDF rat contrasts to the improvement associated with the initiation of insulin treatment of humans with type 2 diabetes. However, as reviewed by Brunzell and Chait,[26] chronic treatment of human type 2 diabetes with insulin often results in persistently elevated levels of VLDL triglyceride along with decreased HDL cholesterol, and these changes are largely independent of glycemic control. The significant obesity maintained in ZDF rats with chronic insulin treatment may be a factor in the sustained hypertriglyceridemia observed in these animals. Obesity in human type 2 diabetes is known to adversely affect diabetic dyslipidemia particularly with regard to increasing VLDL triglyceride and decreasing HDL cholesterol.[28] Indeed, the well-known weight gain associated with use of insulin to achieve glycemic control is a major concern clinically in view of potential adverse effects on lipid status.[1] Thus, chronic insulin treatment of ZDF rats which accentuated VLDL triglyceride levels may resemble the corresponding behavior in some human subjects. However, major differences exist in HDL metabolism between humans and rats. Importantly, there is a lack of cholesteryl ester transfer protein (CETP) in rats[29] and CETP levels influence triglyceride exchange and HDL stability and catabolism. However, the association of increased triglyceride and decreased HDL is well known and this was the result observed in the current study. The mechanism for increases in HDL and apo A-I in the progression of rat diabetes is unknown and deserves further study.

In summary, insulin treatment normalizes glucose levels in ZDF rats, but does not correct the hyperlipidemia, and it appears to accentuate hypertriglyceridemia. These results support the 2-defect hypothesis for the diabetes observed in the ZDF rat model.[4] The first defect is responsible for insulin resistance and the second is responsible for pancreatic insufficiency. Insulin treatment of ZDF rats compensates for the pancreatic insufficiency but not for the insulin resistance associated with the *fa/fa* genotype. Although there is a need to be circumspect when comparing diabetes in rats to their human counterparts, current results point to the need to control more than blood glucose in managing diabetes, paying particular attention to nutritional approaches to maintain ideal body weight.

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