Serum lipids and lipoprotein composition in spontaneously diabetic BB Wistar rats

Suman T. Patel, Howard A. I. Newman, Allan J. Yates, Pierre Thibert, and James M. Falko

Abstract Serum lipid and lipoprotein composition in spontaneously diabetic BB Wistar rats, nondiabetic littersmates, and control Wistar rats was studied to elucidate diabetes-related abnormalities of lipoprotein composition. Serum total triglycerides and pre-B-lipoprotein concentrations of insulin-treated spontaneously diabetic BB and nondiabetic littersmate rats were significantly higher than those of control Wistar rats. Serum cholesterol and HDL cholesterol concentrations of spontaneously diabetic BB and nondiabetic littersmate rats did not differ from controls. Concentrations of very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) of spontaneously diabetic BB and nondiabetic littersmate rats were higher than those of normal rats. With sodium dodecyl sulfate-polyacrylamide gel electrophoresis it was observed that the spontaneously diabetic BB and nondiabetic littersmate rat VLDL contained higher percentages of apoE relative to total apoC when compared with control Wistar rats. With isoelectric focusing, apoC-II relative percentages in VLDL and HDL of both spontaneously diabetic BB and nondiabetic littersmate rats were higher than apoC-II proportions in VLDL and HDL of controls. Apolipoprotein A-I of the control rat HDL showed four isoforms that focused at pI 5.8 (17.3%), 5.75 (30.6%), 5.65 (31.8%), and 5.55 (20.5%); however, the spontaneously diabetic BB and nondiabetic littersmate rat HDL apoA-I was mainly represented by two isoforms that focused at pI 5.8 and 5.75. VLDL of both diabetic and nondiabetic BB rats contained higher levels of acidic apoE isoforms compared to their counterparts in control Wistar rats. Although HDL cholesterol concentrations of spontaneously diabetic BB rats remained normal, protein concentrations were higher resulting in a low cholesterol/protein ratio in HDL suggesting that the cholesterol-carrying capacity of spontaneously diabetic BB rat HDL could be less than normal and may be due to an abnormal apoA-I composition. Quantitative alterations of lipid and lipoprotein composition appear in the BB Wistar rat when compared to the Wistar rat, but some of the changes are more pronounced in the spontaneously diabetic BB Wistar rat.—Patel, S. T., H. A. I. Newman, A. J. Yates, P. Thibert, and J. M. Falko. Serum lipids and lipoprotein composition in spontaneously diabetic BB Wistar rats. J. Lipid Res. 1984. 25: 1072–1083.

Supplementary key words VLDL • HDL • HDL-cholesterol • apoC subunits • apoA-I isoforms • apoE isoforms

The incidence of coronary heart disease is much greater in diabetic than in nondiabetic humans (1, 2) and is related to elevated concentrations of serum lipid and abnormalities of lipoproteins (3–5). Chemically induced diabetes-related modifications in serum lipids and lipoproteins have been investigated in animal models to elucidate the mechanism(s) leading to accelerated atherosclerosis in human diabetic patients (6–9). Bar-On, Roheim, and Eder (10) studied the effects of streptozotocin-induced diabetes on plasma lipids and lipoproteins in the rat. In contrast to the chemically induced diabetic rat model, we used a spontaneously diabetic rat model, the BB (Bio-Breeding) Wistar rat, a promising animal model for human diabetes mellitus (11, 12). The onset of overt diabetes in SDBB occurs at age 40–140 days with a mean of about 90 days, and a careful study of NDLM rats showed that they exhibited glucose intolerance (13). Several pathological changes, some of which are diabetes-related, have been observed in the BB rat (14–21). Subbiah and Deitemeyer (22) reported increased formation of thromboxane in platelets and decreased formation of prostacyclin in the aorta of insulin-treated diabetic BB Wistar rats. Except for the reports on serum total TG and CH (23, 24), there is a paucity of information on serum lipids and lipoproteins of SDBB rats. In the present study we report quantitative differences

Abbreviations: BB, Bio-Breeding; HDL, high density lipoproteins; VLDL, very low density lipoproteins; LDL, low density lipoproteins; TG, triglyceride; CH, cholesterol; SDBB, spontaneously diabetic Bio-Breeding; NDLM, nondiabetic littersmates; LP, lipoprotein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; IEF, isoelectric focusing; alb, albumin.

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in serum lipids and lipoproteins of SDBB, NDLM, and control Wistar rats.

MATERIALS AND METHODS

Chemicals

Acrylamide, N,N'-methylene-bis-acrylamide, riboflavin-5-phosphate, urea, ampholines, and Coomassie Brilliant Blue R-250 were purchased from Bio-Rad Laboratories, Rockville Center, NY. Urea solutions were freshly prepared and passed through a Rexyn-I-500 column to prevent carbamylation of apolipoproteins. A-Gent Cholesterol and A-Gent Triglyceride reagents were from Abbott Laboratories, Diagnostic Division, Chicago, IL. Cholesterol esterase and cholesterol oxidase were obtained from Miles Laboratories, Inc., Elkhart, IN. Agarose plates for electrophoresis were purchased from Corning Medical, Medfield, MA.

Animals and sample collection

Male SDBB rats (n = 20; fourteen, 8 months old and six, 11 months old), nondiabetic littermates (n = 10; 8–9 months old), ten age-matched and ten weight-matched control Wistar rats were used for the study. Rat body weights ranged from 350 to 450 g. The SDBB and NDLM rats were obtained from the Division of Animal Resources, Ottawa, Canada, and control Wistar rats were obtained from Harlan Industries (Indianapolis, IN). Maintenance and sample collection of the animals has been described in detail previously (25). All rats were housed in a semi-barrier housing facility. The animal room was maintained under slight positive pressure at 22°C and supplied with fresh air passed through high-efficiency particle filters at the rate of 16 air changes per hr. Humidity varied between 45% and 55%, and there was a photoperiod with a 12-hr light-dark cycle. All rats had access to Purina rat chow and water ad libitum. SDBB and NDLM rats were weighed and monitored daily for ketones (Ketostix, Ames Co., Elkhart, IN) and glucose (test tape, Eli Lilly Co., Indianapolis, IN). The diabetic rats received daily subcutaneous injections of insulin sufficient to maintain +4 glucosuria and from zero to large amounts of ketones in the urine. Thus, the diabetic animals were maintained in a diabetic state for at least 5 months. Diabetic rats without insulin treatment became highly ketotic, developed hyperlipidemia with serum TG and CH up to 3000 mg/dl and 365 mg/dl, respectively. SDBB rats without insulin treatment died within a few days because of severe diabetic ketoacidosis.

Following an overnight fast (16–18 hr) rats were lightly anesthetized with ether and killed by exsanguination from the abdominal aorta. Blood from individual rats was collected in glass test tubes and allowed to clot at room temperature. Serum was collected following centrifugation at 2000 g for 10 min. Serum total TG, CH, HDL-CH, preβ-LP-TG, β-LP-TG, and α-LP-TG were estimated in a portion of each sample, and the remainder of the serum was subjected to ultracentrifugation for the isolation of lipoproteins. Since lipoprotein analysis required approximately twice the amount of serum available from any one animal, serum from two animals was pooled to yield eight pools from SDBB, five pools from NDLM, and five pools from age-matched control rats. These pools were used for preparative ultracentrifugation to isolate lipoproteins.

Lipoprotein isolation and characterization

Serum was fractionated into lipoproteins of the following density classes by differential ultracentrifugation. Densities were adjusted according to Havel, Eder, and Bragdon (26). Fractions were collected using a Spinco tube slicer. A VLDL fraction with d < 1.006 g/ml, an LDL fraction within the density range 1.006–1.063 g/ml, and an HDL fraction at d 1.063–1.21 g/ml were collected and dialyzed against 0.15 M NaCl–0.005 M EDTA, pH 7.5, at 4°C. The purity of the lipoprotein fractions was checked by agarose gel electrophoresis, and contaminated fractions were readjusted to their respective densities and recentrifuged to obtain purified fractions. The LDL fraction (d 1.006–1.063 g/ml) is known to contain traces of HDL and so was not used for IEF. It is known that isolation of pure LDL requires fractionation of the lipoprotein in the density range 1.019-1.040 g/ml (27). No attempt was made to characterize the fraction collected at d 1.006–1.063 g/ml, and for simplicity this lipoprotein fraction is designated as LDL. Albumin contamination in the fractions was checked by a double-immunodiffusion technique with rat anti-albumin serum. HDL fractions obtained after one centrifugation at d 1.21 g/ml were found to be contaminated. An aliquot of HDL was used for SDS-PAGE without albumin removal. However, for the IEF of apoHDL, albumin was removed from HDL by recentrifugation at d 1.21 g/ml. The protein content of pure VLDL, LDL, and HDL was measured by the method of Lowry et al. (28) as modified by Sata, Havel, and Jones (29). Subsequently, appropriate amounts of VLDL and HDL were lyophilized and delipidated by the methods of Brown, Levy, and Fredrickson (30) and Scanu and Edelstein (31). The apoVLDL and apoHDL were solubilized in 0.01 M Tris buffer, pH 8.2, containing 8 M urea and dithiothreitol (1.54 mg/ml of buffer).

Apolipoprotein electrophoresis

ApoVLDL and apoHDL were separated with SDS-PAGE as described by Shore et al. (32). Following electrophoresis the gels were stained (32) and scanned at 570 nm in a Gilford Model 240 spectrophotometer.
analyses

Serum total CH concentrations were measured by the method of Allain et al. (39) with A-Gent Cholesterol reagent on the Abbott Bichromatic analyzer (ABA-100) with a 1:100 syringe plate. Serum total TG concentrations was measured on the ABA-100 using A-Gent Triglyceride reagent according to the methodology of Sampson, Demers, and Krieg (40). HDL-CH was determined by an enzymatic method after electrophoretic separation of lipoproteins on agarose film as described by Newman and Jenny (41), except samples (2-3 μl) were applied to each well of the agarose plate (5 μl sample was applied when total serum CH was less than 100 mg/dl), and electrophoresis was done with 3-(N-morpholino)-2-hydroxypropane sulfonic acid buffer, pH 7.8 (Corning Medical, Palo Alto, CA).

After separating preβ-LP, β-LP, and α-LP electrophoretically, TG content of the lipoprotein fractions was measured as described by O'Brien et al. (42). TG in lipoproteins separated by electrophoresis was stained by a procedure using the colorimetric method of Stavropoulos and Crouch (43). Two to 3 μl of rat serum was applied to each well of the agarose plate, and electrophoresis was done at 90 V for 25 min with Universal barbital buffer, pH 8.6. Following electrophoresis, plates were overlaid with reconstituted (0.5 ml) Triglyceride Reagent (Dow Chemical Co., Indianapolis, IN). Plates were incubated at 37°C (15 min) and room temperature (15 min) for maximal color development and were scanned using the Corning Model 720 densitometer. The relative percentages of preβ-, β-, and α-LP-TG were determined. LP-TG concentrations were computed by multiplying serum total TG with the relative percentage of LP. This method was compared to the analysis of TG from ultracentrifugally isolated VLDL, LDL, and HDL. Six rat serum pools were obtained from a separate clofibrate study which had six groups of rats. Preβ-LP-TG, β-LP-TG, and α-LP-TG values obtained by the electrophoresis method were 25.3 ± 16.4 mg/dl, 32.2 ± 11.4 mg/dl, and 9.5 ± 2.1 mg/dl, respectively, which compared to the ultracentrifugation values of VLDL-TG (26.8 ± 11.2 mg/dl) and LDL-TG (25.3 ± 3.7 mg/dl). HDL-TG (5.7 ± 0.8 mg/dl) appeared to be significantly lower than α-LP-TG.

Statistical analysis

Differences in the concentration of analytes of SDBB, NDLM, and control animals were evaluated statistically by ANOVA followed by Duncan's test (44) or the Kruskal and Wallis rank sum test (45). "t" distributions of arc sine √x of apolipoprotein percentages were also calculated.

| TABLE 1. Serum total triglyceride and cholesterol concentrations of male age-matched Wistar controls, weight-matched Wistar controls, nondiabetic littermates, and diabetic BB Wistar rats

<table>
<thead>
<tr>
<th>Serum Triglycerides</th>
<th>Serum Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/dl</td>
<td></td>
</tr>
<tr>
<td>Age-matched controls (n = 10)</td>
<td>58.5 ± 4.2</td>
</tr>
<tr>
<td>Weight-matched controls (n = 10)</td>
<td>61.4 ± 6.7</td>
</tr>
<tr>
<td>Nondiabetic littermates (n = 10)</td>
<td>136 ± 38.7</td>
</tr>
<tr>
<td>SDBB rats (n = 10)</td>
<td>154 ± 47.8</td>
</tr>
</tbody>
</table>

* Values represent mean ± SD.

* Significantly different from their age-matched and weight-matched controls (P < 0.005).
TABLE 2. Protein and triglyceride composition of VLDL and LDL of male age-matched Wistar controls, weight-matched Wistar controls, nondiabetic littermates, and diabetic BB Wistar rats

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Protein</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preβ-LP</td>
<td>16.0 ± 1.8</td>
<td>34.8 ± 10.8</td>
</tr>
<tr>
<td>LDL</td>
<td>22.6 ± 3.3</td>
<td>9.8 ± 2.1</td>
</tr>
<tr>
<td>Weight-matched controls (n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preβ-LP</td>
<td>37.2 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>40.2 ± 6.6</td>
<td>13.6 ± 4.6</td>
</tr>
<tr>
<td>Nondiabetic littermates (n = 5 pools from 10 control Wistar rats)</td>
<td>23.6 ± 1.1</td>
<td>92.7 ± 40.8</td>
</tr>
<tr>
<td>Preβ-LP</td>
<td>40.2 ± 6.6</td>
<td>13.6 ± 4.6</td>
</tr>
<tr>
<td>LDL</td>
<td>38.8 ± 2.6</td>
<td>14.3 ± 6.0</td>
</tr>
<tr>
<td>SDBB rats (n = 10)</td>
<td>22.7 ± 6.9</td>
<td>108 ± 48.5</td>
</tr>
<tr>
<td>Preβ-LP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Values represent mean ± SD.
b For protein measurement, n = 5 pools from 10 control Wistar rats; for triglycerides, n = 10.
c The analyte was not measured.
d For protein measurement, n = 5 pools from 10 nondiabetic littermates; for triglycerides, n = 10.
Significantly different from controls (P < 0.005).

RESULTS

The data on serum lipid and lipoprotein compositions of SDBB, NDLM, and control rats are shown in Table 1, Table 2, and Table 3. The serum total TG concentrations in both SDBB and NDLM rats were significantly higher (P < 0.005) than those of both age- and weight-matched control Wistar rats. However, serum TG concentrations of SDBB rats did not differ significantly from their nondiabetic littermates. The hypertriglyceridemia in SDBB rats and their littermates was due to the higher preβ-LP-TG concentrations which were significantly greater (P < 0.005) than those of other nondiabetic control groups (Table 2). β-LP-TG and α-LP-TG concentrations of SDBB and NDLM rats did not differ significantly from controls. Comparison of concentrations of serum CH and HDL-CH in all the groups of rats showed no significant differences (Tables 1 and 3). SDBB and NDLM rats had significantly higher VLDL, LDL, and HDL protein concentrations than control Wistar rats (P < 0.005); HDL protein content of SDBB rats was higher than that of NDLM rats (Tables 2 and 3).

SDS-PAGE of VLDL apolipoproteins showed a separate apoE band and two apoC's which were not separated clearly (Fig. 1A), resulting in poorly separated peaks on the tracing in this area (Fig. 1B). ApoVLDL did not show any apoA-IV band, probably because fasting samples without chylomicrons were used for the isolation of lipoproteins. This observation differs from that of Bar-On et al. (10) who reported that apoA-IV increased in diabetic rats. However, in studies of these authors, VLDL-TG levels of diabetic rats were >1000 mg/dl.

The percentage of apoE relative to total apoC in VLDL of SDBB and NDLM rats was significantly higher than that found in VLDL of control Wistar rats (Table 4).

TABLE 3. Protein, cholesterol, and triglyceride content of HDL of male age-matched Wistar controls, weight-matched Wistar controls, nondiabetic littermates, and diabetic BB Wistar rats

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Protein</th>
<th>Cholesterol</th>
<th>α-LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preβ-LP</td>
<td>68.8 ± 13.5</td>
<td>58.0 ± 11.6</td>
<td>11.2 ± 2.1</td>
</tr>
<tr>
<td>LDL</td>
<td>54.0 ± 11.6</td>
<td>12.7 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>Weight-matched controls (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preβ-LP</td>
<td>64.3 ± 11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>12.7 ± 6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic littermates (n = 5 pools from 10 control Wistar rats)</td>
<td>80.4 ± 6.7</td>
<td>74.9 ± 13.6</td>
<td></td>
</tr>
<tr>
<td>Preβ-LP</td>
<td>64.3 ± 11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>12.7 ± 6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDBB rats (n = 10)</td>
<td>109.4 ± 9.3</td>
<td>61.5 ± 14.5</td>
<td></td>
</tr>
<tr>
<td>Preβ-LP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Values represent mean ± SD.
b Number of pools for each group, same as in Table 2.
c The analyte was not measured.
d Significantly different from SDBB (P < 0.05).
e Significantly different from controls (P < 0.005) and NDLM (P < 0.05).
Fig. 1. Photographs of SDS-PAGE gels (A) and densitometric scans (B) of control (a), NDLM (b), and SDBB (c) rat VLDL apolipoproteins.

As a result, the ratio of apoE to apoC in the SDBB and NDLM rat LDL was also significantly higher ($P < 0.005$).

Typical IEF distribution patterns and scans of apoVLDL (Fig. 2A and B) showed the same basic distribution pattern as reported by Gidez et al. (37). ApoC peptides of VLDL were focused in the pH range 4.43–4.74, which showed five bands after staining; these are characterized as apoC-II (pH 4.74), apoC-III-0 (pH 4.67), apoC-III-2(-1) (pH 4.61), apoC-III-3 (pH 4.5), and apoC-III-4 (pH 4.43) in order of decreasing pH. The proportions of apoC-II in apoVLDL of SDBB and NDLM rats were found to be almost double those in apoVLDL of control Wistar rats, a significant increase ($P < 0.005$, Table 5). The percentages of apoC-III-2(-1) in SDBB rat VLDL apoC were also found to be significantly higher than those of controls ($P < 0.05$). The proportions of apoC-III-0 and apoC-III-3 in apoVLDL of SDBB and NDLM rats were found to be lower than those in the control Wistar rats. When corrected for higher chromogenicity of apoC-II, apoC-II/apoC-III ratios of VLDL of SDBB (0.24 ± 0.02) and NDLM (0.21 ± 0.01) rats were higher than those of controls (0.10 ± 0.01), $P < 0.005$. ApoE of VLDL focused between pH 5.31 and 5.46. Both the SDBB and NDLM rat VLDL showed relatively higher percentages of apoE-2 resulting in a lower apoE-3/apoE-2 ratio when compared with that of the control Wistar rat HDL ($P < 0.005$, Table 6).

Both distribution patterns and scans of apoHDL (Fig. 3A and B) demonstrate well separated apoA-I, apoE, apoA-IV, and two apoC bands; apoA-II was not clearly visible. An unidentified band below apoA-I was observed on the SDS-PAGE gel of apoHDL. Similarly, Connelly and Kuksis (46) also reported the unidentified band on SDS-glycerol-PAGE of rat apoHDL. Despite the higher ratio of apoE/apoC in VLDL of SDBB and NDLM rats, the SDBB and NDLM rat HDL had a lower apoE/apoC ratio indicating a relatively higher apoC content (Table 7). Levels of apoA-IV in HDL of SDBB rats were significantly lower than those of controls. ApoC percentages used in the computation of apoE/apoC ratio include all apoC subspecies as well as one-half of the apoA-II. The present finding, although semiquantitative, compares with quantitative observations reported by Bar-On et al. (10) with streptozotocin-treated rats.

Typical IEF distribution patterns of apoHDL from the three different groups of rats (Fig. 4A and B) were

| Table 4: SDS-PAGE distribution of apoE and apoC (apoC-II + C-III + C-I) in VLDL of control Wistar rats, non-diabetic littersmates, and SDBB rats |
|-----------------|----------------|----------------|
|                 | ApoE           | ApoC           |
|                 | $\%$ of total  | (Total)        |
| Age-matched control$^b$ (n = 5) | 15.5 ± 1.0 | 84.3 ± 1.2 | 0.19 ± 0.02 |
| Nondiabetic littersmates$^c$ (n = 5) | 25.6 ± 1.2$^f$ | 74.4 ± 1.2$^f$ | 0.34 ± 0.02$^f$ |
| SDBB rats$^d$ (n = 8) | 24.6 ± 0.8$^f$ | 75.4 ± 0.9$^f$ | 0.33 ± 0.02$^f$ |

$^a$ Values represent mean ± SD.
$^b$ n = 5, Pools from 10 control Wistar rats.
$^c$ n = 5, Pools from 10 non-diabetic littersmates.
$^d$ Significantly different from controls ($P < 0.005$).
$^e$ n = 8, Pools from 20 SDBB rats.
observed with the first four bands (pI 5.55 to 5.8) from the top in the apoA-I region distinctly separated from the apoA-IV and apoE region (pI 5.3 to 5.46), allowing a comparison of protein distributions among the four well separated apoA-I isoforms. ApoA-IV and E isoforms from different groups of rats were not compared because of the lack of clear separation of apoA-IV from apoE on IEF gels. ApoA-I-2 and apoA-I-3 were the major isoforms of apoA-I in HDL of control Wistar rats, whereas HDL apoA-I of the SDBB and NDLM rats was represented mainly by apoA-I-1 (pI 5.8) and apoA-I-2 (pI 5.75), with lower proportions of apoA-I-3 (pI 5.65) and apoA-I-4 (pI 5.55, Table 8).

The proportions of apoC-II in apoHDL of SDBB and NDLM rats were higher than those in apoHDL of normal Wistar rats (Table 9). Conversely, the apoC-III-

TABLE 5. Distribution pattern of apoC-II and C-III in VLDL of control Wistar rats, nondiabetic littermates and SDBB ratsd

<table>
<thead>
<tr>
<th></th>
<th>apoC-II</th>
<th>apoC-II-0</th>
<th>apoC-II-2(1)</th>
<th>apoC-II-3</th>
<th>apoC-II-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(pI 4.74)</td>
<td>(pI 4.67)</td>
<td>(pI 4.61)</td>
<td>(pI 4.5)</td>
<td>(pI 4.43)</td>
</tr>
<tr>
<td>Controls age-matchedb (n = 5)</td>
<td>11.1 ± 1.0</td>
<td>33.7 ± 2.9</td>
<td>9.9 ± 1.6</td>
<td>38.3 ± 3.6</td>
<td>6.9 ± 0.8</td>
</tr>
<tr>
<td>Nondiabetic littermatesb (n = 5)</td>
<td>20.7 ± 1.4</td>
<td>28.6 ± 3.2</td>
<td>12.8 ± 1.7d</td>
<td>33.0 ± 2.5c</td>
<td>4.2 ± 2.3d</td>
</tr>
<tr>
<td>SDBB ratsd (n = 8)</td>
<td>24.2 ± 2.1c</td>
<td>25.4 ± 1.9c</td>
<td>16.5 ± 2.3c</td>
<td>29.9 ± 1.1c</td>
<td>3.8 ± 0.5c</td>
</tr>
</tbody>
</table>

% of total

a Values represent mean ± SD.

b Number of pools for each group, same as in Table 4.

c Significantly different from controls (P < 0.005).

d Significantly different from controls (P < 0.05).
TABLE 6. Distribution of VLDL apoE isoforms of control Wistar rats, nondiabetic littermates, and SDBB rats (expressed as percentages)

<table>
<thead>
<tr>
<th>Age-matched controlb</th>
<th>ApoE-1</th>
<th>ApoE-2</th>
<th>ApoE-3</th>
<th>ApoE-4</th>
<th>ApoE-3/ ApoE-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22.3 ± 1.4</td>
<td>10.0 ± 0.7</td>
<td>61.9 ± 5.5</td>
<td>5.7 ± 0.5</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Nondiabetic littermatesb</td>
<td>18.6 ± 1.2</td>
<td>18.4 ± 1.0f</td>
<td>56.9 ± 5.2</td>
<td>6.0 ± 0.5</td>
<td>3.1 ± 0.4f</td>
</tr>
<tr>
<td>SDBB ratsb</td>
<td>19.0 ± 0.7</td>
<td>15.8 ± 1.2f</td>
<td>57.8 ± 4.9</td>
<td>6.8 ± 1.1</td>
<td>3.6 ± 0.4f</td>
</tr>
</tbody>
</table>

Values represent mean ± SD.

Number of pools for each group, same as in Table 4.

Significantly different from controls (P < 0.005).

0 proportions were lower in SDBB and NDLM rat apoHDL. There was no difference in the percentage distribution of apoC-III-2(-1) among the three groups. However, apoC-III-4 was significantly higher in the control apoHDL and the percentage of apoC-III-3 was higher in SDBB rats.

**DISCUSSION**

This study shows that hypertriglyceridemia and profound changes of apolipoprotein distribution pattern occur in both spontaneously diabetic SDBB rats and their nondiabetic littermates. The possibility that changes in lipid concentrations and apolipoprotein distribution are strain-related rather than disease-related is further supported by the findings of a commonality of pathological changes in organs (18, 20) of both SDBB and NDLM rats. Although insulin-treated adult SDBB and NDLM rats had higher fasting serum TG concentrations compared to control Wistar rats, there was no elevation of serum CH levels in either of these two groups. However, elevations of both serum TG and CH have been reported in streptozotocin-induced diabetic rats (10, 47, 48). The high serum TG concentration in SDBB rats was represented mainly by preβ-LP-TG, a finding also similar to the one reported for streptozotocin-induced diabetic rats (10, 47). Serum CH and HDL-CH concentrations of SDBB and their nondiabetic littermates did not differ significantly from those of control Wistar rats. However, Bar-On and Eisenberg (49) reported increased HDL-CH concentrations following the chemical induction of diabetes in rats. Lack of consistent findings for relationships between diabetes and HDL-CH have been reported for human diabetic subjects and attributed to the heterogeneity of the diabetic populations under investigation and to their treatments (2). It has been reported that insulin-deficient or poorly controlled insulin-requiring diabetics have low HDL-CH levels (50–52). Higher concentrations of protein in SDBB and NDLM rat lipoprotein fractions could
TABLE 7. SDS-PAGE distribution pattern of HDL apolipoproteins of control Wistar rats, nondiabetic littermates, and SDBB rats (expressed as percentages)

<table>
<thead>
<tr>
<th>Age-matched control</th>
<th>ApoE</th>
<th>ApoA-IV</th>
<th>ApoA-I</th>
<th>ApoC's</th>
<th>ApoE/ApoC (Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.1 ± 0.5</td>
<td>11.6 ± 1.3</td>
<td>58.4 ± 5.5</td>
<td>21.6 ± 3.5</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Nondiabetic littermates</td>
<td>4.2 ± 0.6</td>
<td>8.9 ± 1.7</td>
<td>53.5 ± 4.1</td>
<td>29.2 ± 2.4 c</td>
<td>0.14 ± 0.03 d</td>
</tr>
<tr>
<td>SDBB rats</td>
<td>5.3 ± 0.5</td>
<td>9.1 ± 0.9 c</td>
<td>52.2 ± 5.6</td>
<td>29.6 ± 3.1 c</td>
<td>0.18 ± 0.02 d</td>
</tr>
</tbody>
</table>

* Values represent mean ± SD.

a Number of pools for each group, same as in Table 4.

b Significantly different from controls (P < 0.005).
c Significantly different from controls (P < 0.05).
d Significantly different from controls (P < 0.05).

can be attributed to an absolute increase in VLDL catabolism with concomitant transfer of apolipoproteins to LDL and HDL pools, resulting from high serum VLDL concentrations as suggested for streptozotocin-induced diabetic rats (10).

The distribution pattern of apoC in VLDL of control fasted rats (this study) is similar to those of Witztum and Schonfeld (53). Also, our observations regarding the distribution pattern of apoC II and apoC III-0 in VLDL of SDBB and NDLM rats are similar to those reported by Bar-On et al. (10). However, they used streptozotocin-treated sucrose-fed rats that were not fasted before collection of blood, and they found higher levels of apoC III-3 in the diabetic rat VLDL. In our study it was observed that insulin-treated and fasted SDBB rats did not show an increase of VLDL apoC III-3. Such discrepancies between our observations and those of Bar-On et al. (10) could be attributed to differences in diet, insulin treatment, and diabetes model.

ApoC II is an activator of lipoprotein lipase and is essential for maximal hydrolysis of VLDL-TG (54–56). Since apoC III inhibits lipoprotein lipase in vitro (57), the increased ratio of apoC II to apoC III may increase lipoprotein lipase activity. We observed hypertriglyceri-
TABLE 8. Distribution of apoA-I isoforms in HDL of control Wistar rats, nondiabetic littermates, and insulin-treated SDBB rats

<table>
<thead>
<tr>
<th>Isoforms of ApoA-I as % of Total apoA-I</th>
<th>Age-matched control&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nondiabetic littermates&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SDBB rats&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1-1 (pI 5.8)</td>
<td>17.3 ± 2.4</td>
<td>41.6 ± 1.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>40.1 ± 2.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>A-1-2 (pI 5.75)</td>
<td>30.6 ± 1.8</td>
<td>33.0 ± 0.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>37.6 ± 2.7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>A-1-3 (pI 5.65)</td>
<td>31.3 ± 2.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16.4 ± 1.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.5 ± 2.3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>A-1-4 (pI 5.55)</td>
<td>20.8 ± 1.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.0 ± 0.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.79 ± 1.33&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values represent mean ± SD.
<sup>b</sup> Number of pools for each group, same as in Table 4.
<sup>c</sup> Significantly different from control (P < 0.005).

idemia in both SDBB and NDLM rats despite higher apoC-II/apoC-III ratios in VLDL of these animals. Although it is too early to draw direct comparisons between SDBB and human diabetes, these points should be noted: a) levels of apoC-II in VLDL of human diabetics were found to be higher than those of nondiabetic controls (58), b) a lipoprotein fraction (d < 1.006 g/ml) of a hypertriglyceridemic patient carried excessive apoC-II but failed to act as an effective substrate for lipoprotein lipase (59, 60). High proportions of apoC-II compared to apoC-III in the SDBB rat VLDL possibly could be a physiological compensatory mechanism to establish TG homeostasis. For a more specific explanation of SDBB and NDLM rat hypertriglyceridemia, it will be necessary to know quantitative levels of C apolipoproteins and their interactions with lipoprotein lipase in these rats. Levels of apoE-3 in VLDL of SDBB and NDLM rats were low when compared to those of controls. We found higher levels of acidic apoE-2 isoforms in VLDL of both SDBB and NDLM rats, a finding similar to the observations reported for type II human diabetic patients (61).

In the present study, control Wistar rat HDL showed four isoforms of apoA-I. Gidez et al. (37) also reported four isoforms of apoA-I in HDL of sucrose-fed rats. However, Sliwkowski, Wu, and Windmueller (62) demonstrated three isoforms of apoA-I in Osborne-Mendel rats and reported that the newly synthesized acidic isoform (apoA-I-4, pI 5.55) converted to the basic isoform (apoA-I-3, pI 5.68). We noticed that both SDBB and NDLM rat HDL contained higher percentages of basic apoA-I isoforms (A-1-1 and A-1-2). The predominance of basic apoA-I isoforms observed in SDBB and NDLM rats could be due to altered conversion of acidic isoforms to basic apoA-I isoforms. Zannis et al. (63-65) have shown an abnormal distribution of apoA-I isoforms in Tangier patients, and an apoA-I Milano variant was demonstrated in an A-IM family (66, 67). Both of these variations in apoA-I were associated with decreased HDL-CH and abnormal lipid binding properties (66, 68-70). Interestingly, the CH:protein ratios (calculated from the data in Table 3) of the SDBB rat HDL were found to be lower (0.56 ± 0.05) than that of controls (0.84 ± 0.2), indicating that the HDL particles of SDBB rats, although rich in protein, carry less CH. This finding may be related to changes in distribution of apoA-I isoforms of SDBB rats.

In summary, marked alterations were observed in

<table>
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<tr>
<th>Table 9. Distribution pattern of apoC-II and C-III in HDL of control Wistar rats, nondiabetic littermates, and SDBB rats&lt;sup&gt;a&lt;/sup&gt;</th>
<th>C Apoproteins in HDL</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-I1 (pI 4.74)</td>
<td>C-I1-0</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Age-matched controls&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.9 ± 1.5</td>
<td>48.2 ± 3.1</td>
</tr>
<tr>
<td>Nondiabetic littermates&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.0 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.3 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SDBB rats&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.0 ± 2.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.8 ± 2.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values represent mean ± SD.
<sup>b</sup> Number of pools for each group, same as in Table 4.
<sup>c</sup> Significantly different from controls (P < 0.005).
<sup>d</sup> Significantly different from controls (P < 0.05).
lipid and lipoprotein composition of the BB Wistar rat. Higher percentages of VLDL apoC-II, acidic apoE isoforms in VLDL, and basic apoA-I isoforms in HDL were associated with hypertriglyceridemia in the BB Wistar rat strain. Since newly synthesized apoA-I and apoE in rats consist predominantly of acidic isoforms, further investigation of the metabolism of apoA-I and apoE isoforms as related to hyperlipoproteinemia of BB Wistar rats seems warranted.

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