

Serum lipids and lipoprotein composition in spontaneously diabetic BB Wistar rats

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Abstract Serum lipid and lipoprotein composition in spontaneously diabetic BB Wistar rats, nondiabetic littermates, and control Wistar rats was studied to elucidate diabetes-related abnormalities of lipoprotein composition. Serum total triglycerides and pre- β -lipoprotein concentrations of insulin-treated spontaneously diabetic BB and nondiabetic littermate rats were significantly higher than those of control Wistar rats. Serum cholesterol and HDL cholesterol concentrations of spontaneously diabetic BB and nondiabetic littermate 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 littermate rats were higher than those of normal rats. With sodium dodecylsulfate-polyacrylamide gel electrophoresis it was observed that the spontaneously diabetic BB and nondiabetic littermate 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 littermate 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 littermate 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 littermates; 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-300 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 treatment 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 was 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, pre β -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

(Gilford Instrument Laboratories, Inc., Oberlin, OH) equipped with a Gilford model 2410-S linear transport and the Sargent-Welch recorder model SRG (Sargent-Welch Scientific Co., Skokie, IL). The percentage of the total area under each peak was computed for each apolipoprotein by tracing the curve (generated from the Gilford) on the Apple Graphics Tablet and computing percent of total area for each peak with a BASIC program run on the Apple II computer.

ApoVLDL and apoHDL were subjected to IEF in 7.5% polyacrylamide gel containing 8 M urea and 2% ampholines, pH 3.5 to 10, as described by Weidman et al. (33). The amount of apolipoprotein used for IEF ranged from 100 to 400 μ g. These amounts of protein loads for IEF of rat apoVLDL and apoHDL were based on an IEF study demonstrating linearity of the dye binding in the 100–400- μ g range. After IEF, the pH gradient along the gel was measured with the Bio-Rad combination microelectrode and pH profiler. Gels were stained with Coomassie Brilliant Blue R-250 and destained as described by Vesterberg and Hansen (34). The gels were scanned at 570 nm and areas under peaks were integrated as described above. To determine if there was a different extent of the dye binding for apoC subspecies, we focused 8–32 μ g of individual human apoC-II and apoC-III subunits and stained them by the methods described above. The dye uptake by individual apolipoproteins was linear; however, apoC-II chromogenicity was 1.26 times greater than that of apoC-III. Our value for the chromogenicity factor of apoC-II is almost identical to the one reported by Catapano et al. (35). Since the rat and human apoC subunits are analogous (36), rat apoC's could have differential chromogenicity similar to their human counterparts. Thus, the chromogenicity factor determined with human apoC-II and C-III was used to correct the rat apoC-II to apoC-III ratios. Stained bands of apoVLDL and apoHDL were characterized according to the system of Gidez, Swaney, and Murnane (37) and Swaney and Gidez (38).

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 concentration 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 μ l) were applied to each well of the agarose plate (3 μ l 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 pre β -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 μ l 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 pre β -, 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. Pre β -LP-TG, β -LP-TG, and α -LP-TG values obtained by the electrophoresis method were 25.3 \pm 16.4 mg/dl, 32.2 \pm 11.4 mg/dl, and 9.5 \pm 2.1 mg/dl, respectively, which compared to the ultracentrifugation values of VLDL-TG (26.8 \pm 11.2 mg/dl) and LDL-TG (25.3 \pm 3.7 mg/dl). HDL-TG (5.7 \pm 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 \sqrt{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^a

	Serum Triglycerides	Serum Cholesterol
	<i>mg/dl</i>	
Age-matched controls (n = 10)	58.5 \pm 4.2	79.8 \pm 13.4
Weight-matched controls (n = 10)	61.4 \pm 6.7	83.8 \pm 13.7
Nondiabetic littermates (n = 10)	136 \pm 38.7 ^b	101 \pm 9.6
SDBB rats (n = 10)	134 \pm 47.8 ^b	102 \pm 16.4

^a Values represent mean \pm SD.

^b 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^a

	VLDL Protein	Pre β -LP Triglyceride	LDL Protein	β -LP Triglyceride
	<i>mg/dl</i>			
Age-matched controls ^b	16.0 \pm 1.8	34.8 \pm 10.8	22.6 \pm 3.3	9.8 \pm 2.1
Weight-matched controls (n = 10)	— ^c	37.2 \pm 7.2	— ^c	11.8 \pm 2.9
Nondiabetic littermates ^d	23.6 \pm 1.1 ^e	92.7 \pm 40.8 ^e	40.2 \pm 6.6 ^e	13.6 \pm 4.6
SDBB rats ^f	22.7 \pm 6.9 ^e	108 \pm 48.5 ^e	38.8 \pm 2.6 ^e	14.3 \pm 6.0

^a Values represent mean \pm 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.

^e Significantly different from controls ($P < 0.005$).

^f For protein measurement, n = 8 pools from 20 SDBB rats; for triglycerides, n = 10.

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 pre β -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^a

	Protein	Cholesterol	α -LP Triglycerides
	<i>mg/dl</i>		
Age-matched control ^b	68.8 \pm 13.3	58.0 \pm 11.6	11.2 \pm 2.1
Weight-matched control ^b (n = 10)	— ^c	64.3 \pm 11.3	12.7 \pm 6.2
Nondiabetic littermates ^b	80.4 \pm 6.7 ^d	74.9 \pm 13.6	16.9 \pm 3.5
SDBB rats ^b	109.4 \pm 9.3 ^e	61.5 \pm 14.5	17.1 \pm 7.3

^a Values represent mean \pm 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 ($P < 0.05$).

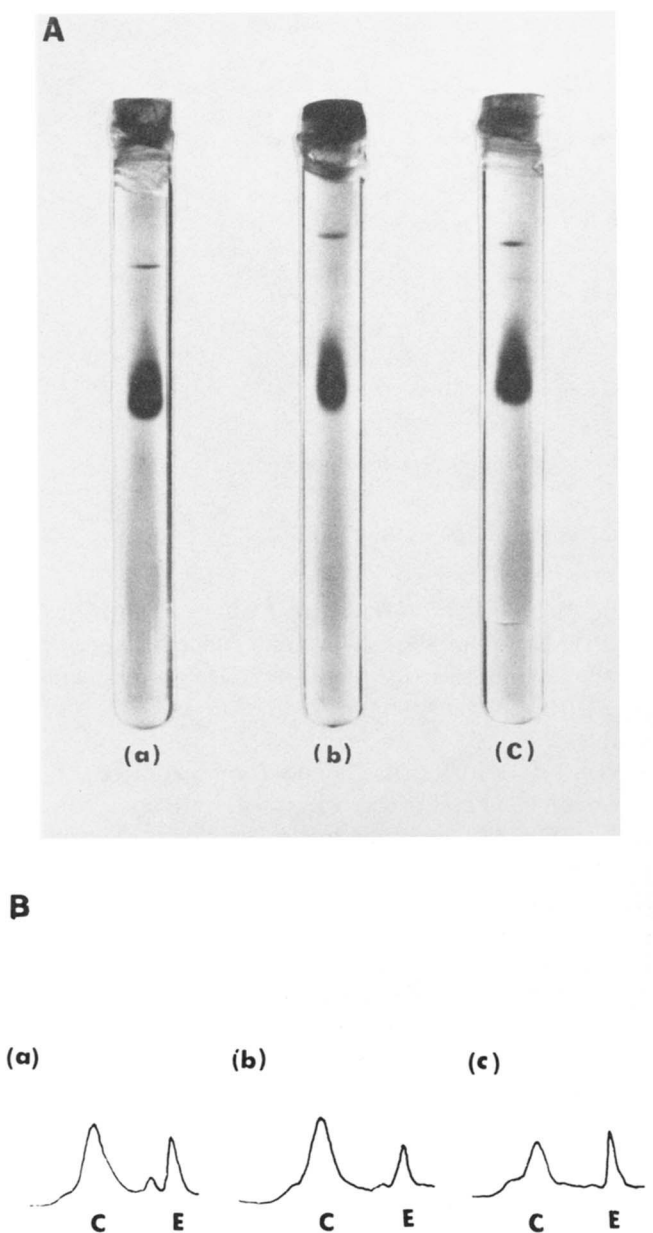


Fig. 1. Photographs of SDS-PAGE gels (A) and densitometric scans (B) of control (a), NDLM (b), and SDBB (c) rat VLDL apolipoproteins.

As a result, the ratio of apoE to apoC in the SDBB and NDLM rat VLDL 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 pI range 4.43–4.74, which showed five bands after staining; these are characterized as apoC-II (pI 4.74), apoC-III-0 (pI 4.67), apoC-III-2(-1) (pI 4.61), apoC-III-3 (pI 4.5), and apoC-

III-4 (pI 4.43) in order of decreasing pI. 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 pI 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-1, 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, nondiabetic littermates, and SDBB rats^a

	ApoE	ApoC	ApoE/ApoC (Total)
	% of total		
Age-matched control ^b (n = 5)	15.5 ± 1.0	84.3 ± 1.2	0.19 ± 0.02
Nondiabetic littermates ^c (n = 5)	25.6 ± 1.2 ^d	74.4 ± 1.2 ^d	0.34 ± 0.02 ^d
SDBB rats ^e (n = 8)	24.6 ± 0.8 ^d	75.4 ± 0.9 ^d	0.33 ± 0.02 ^d

^a Values represent mean ± SD.

^b n = 5, Pools from 10 control Wistar rats.

^c n = 5, Pools from 10 nondiabetic littermates.

^d Significantly different from controls ($P < 0.005$).

^e n = 8, Pools from 20 SDBB rats.

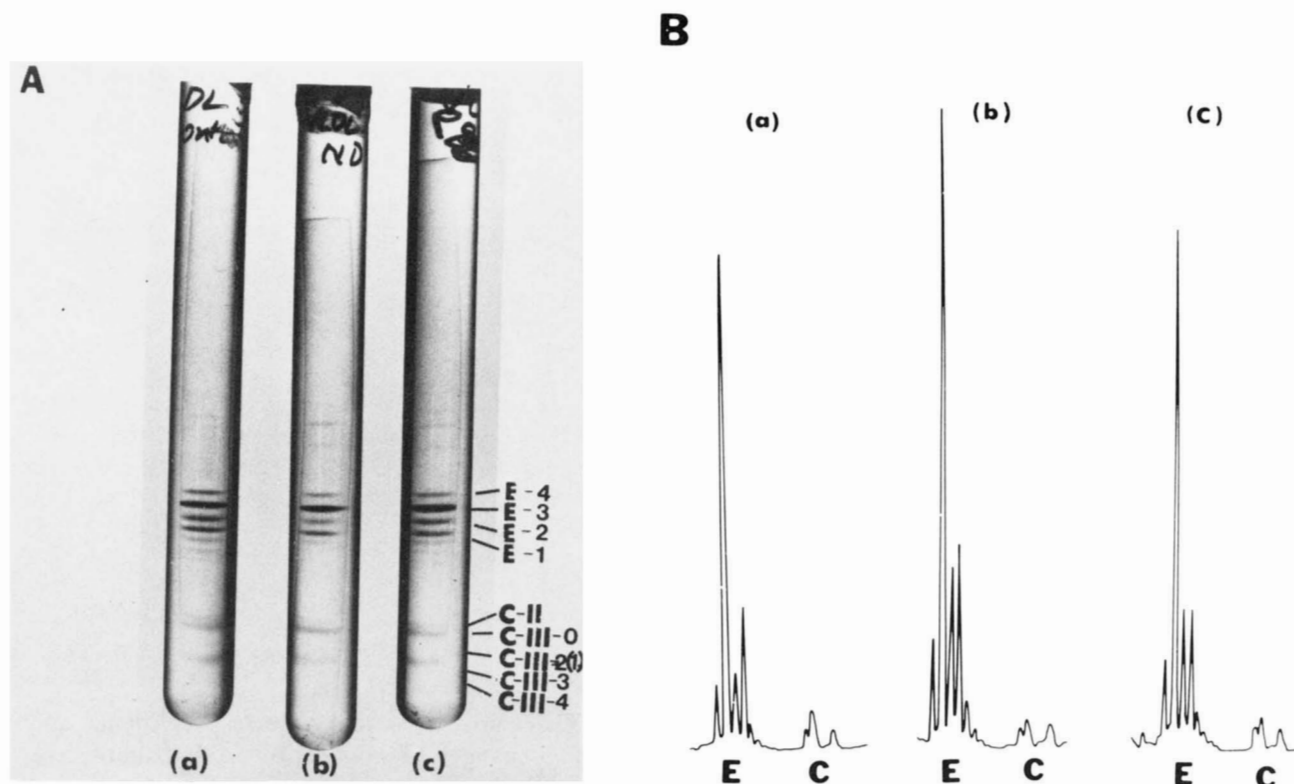


Fig. 2. Photographs of IEF gels (A) and densitometric scans (B) of control (a), NDLM (b), and SDBB (c) rat VLDL apolipoproteins.

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 rats^a

	C Apoproteins in VLDL				
	C-II (pI 4.74)	C-III-0 (pI 4.67)	C-III-2(1) (pI 4.61)	C-III-3 (pI 4.5)	C-III-4 (pI 4.43)
	% of total				
Controls age-matched ^b (n = 5)	11.1 ± 1.0	33.7 ± 2.9	9.9 ± 1.6	38.3 ± 3.6	6.9 ± 0.8
Nondiabetic littermates ^b (n = 5)	20.7 ± 1.4 ^c	28.6 ± 3.2 ^c	12.8 ± 1.7 ^d	33.0 ± 2.5 ^c	4.2 ± 2.3 ^d
SDBB rats ^b (n = 8)	24.2 ± 2.1 ^c	25.4 ± 1.9 ^c	16.5 ± 2.3 ^c	29.9 ± 1.1 ^c	3.8 ± 0.5 ^c

^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)^a

	ApoE-1	ApoE-2	ApoE-3	ApoE-4	ApoE-3/ ApoE-2
Age-matched control ^b	22.3 ± 1.4	10.0 ± 0.7	61.9 ± 5.5	5.7 ± 0.5	6.2 ± 0.1
Nondiabetic littermates ^b	18.6 ± 1.2	18.4 ± 1.0 ^c	56.9 ± 5.2	6.0 ± 0.5	3.1 ± 0.4 ^c
SDBB rats ^b	19.0 ± 0.7	15.8 ± 1.2 ^c	57.8 ± 4.9	6.8 ± 1.1	3.6 ± 0.4 ^c

^a Values represent mean ± SD.

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

^c Significantly different from controls ($P < 0.005$).

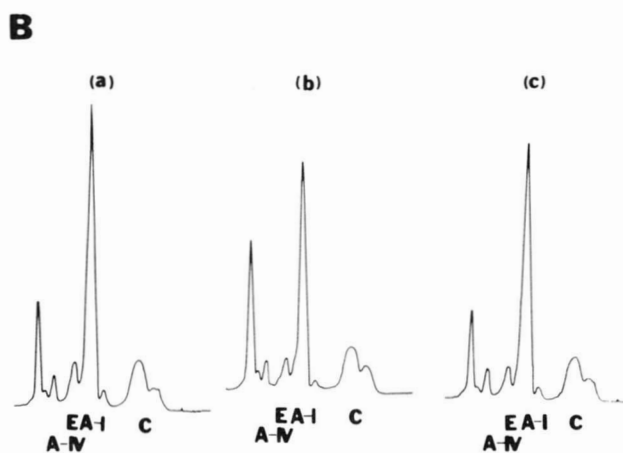
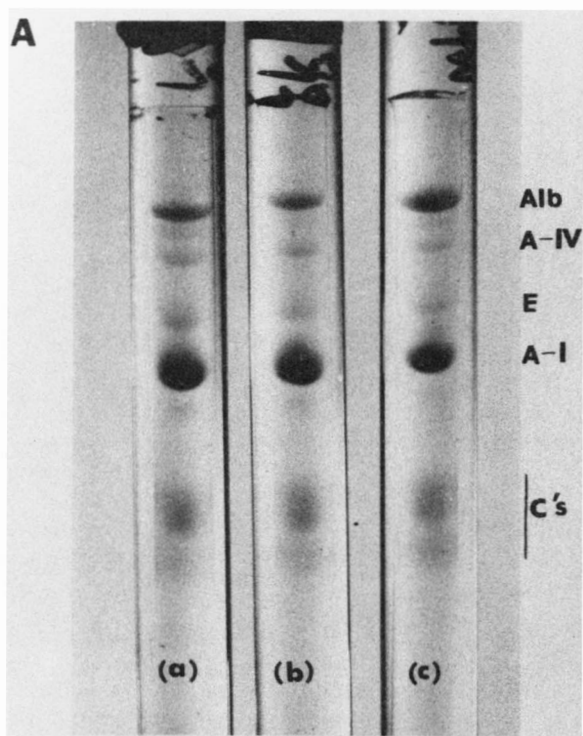


Fig. 3. Photographs of SDS-PAGE gels (A) and densitometric scans (B) of control (a), NDLM (b), and SDBB (c) rat HDL apolipoproteins.

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 pre β -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)^a

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

^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 ($P < 0.05$).

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 hypertriglycer-

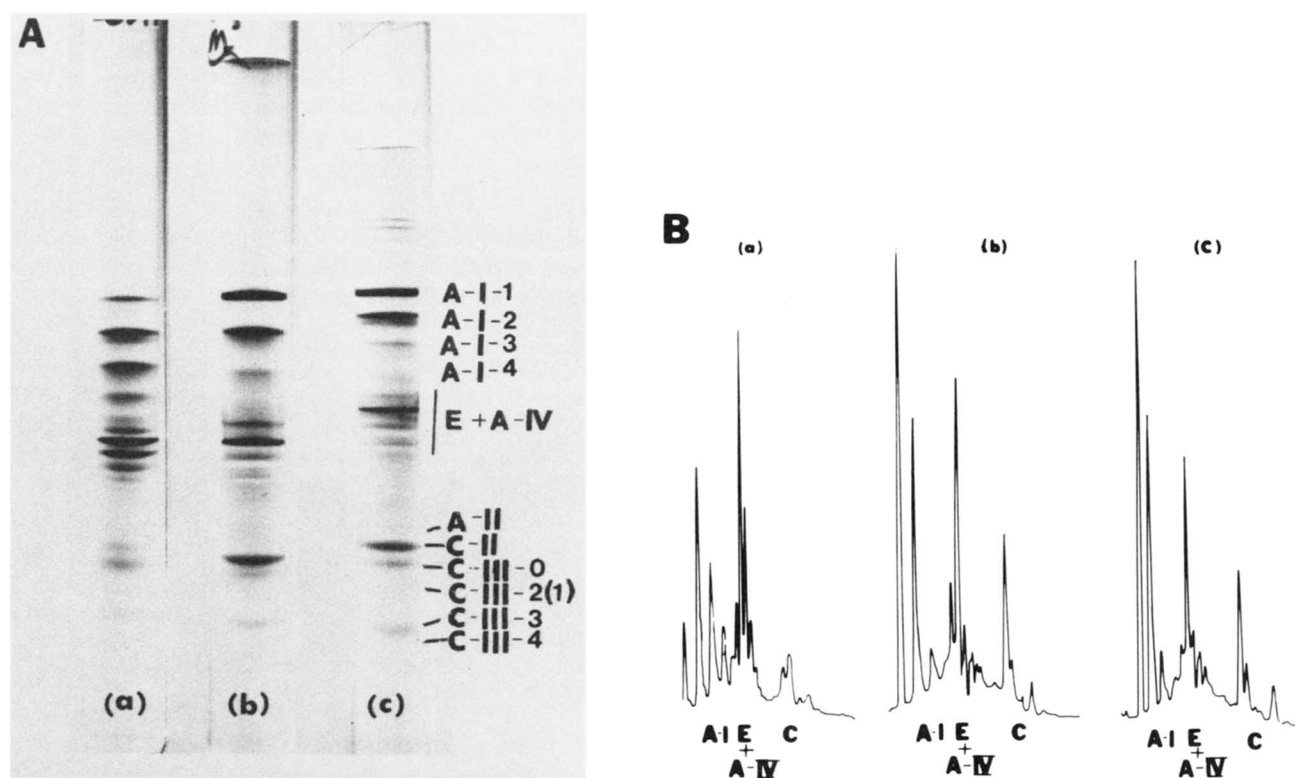


Fig. 4. Photographs of IEF gels (A) and densitometric scans (B) of control (a), NDLM (b), and SDBB (c) rat HDL apolipoproteins.

TABLE 8. Distribution of apoA-I isoforms in HDL of control Wistar rats, nondiabetic littermates, and insulin-treated SDBB rats^a

	Isoforms of ApoA-I as % of Total apoA-I			
	A-I-1 (pI 5.8)	A-I-2 (pI 5.75)	A-I-3 (pI 5.65)	A-I-4 (pI 5.55)
Age-matched control ^b	17.3 ± 2.4	30.6 ± 1.8	31.3 ± 2.5	20.8 ± 1.9
Nondiabetic littermates ^b	41.6 ± 1.9 ^c	33.0 ± 0.8 ^c	16.4 ± 1.3 ^c	9.0 ± 0.8 ^c
SDBB rats ^b	46.1 ± 2.5 ^c	37.6 ± 2.7 ^c	11.5 ± 2.3 ^c	4.79 ± 1.33 ^c

^a Values represent mean ± SD.

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

^c 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, Sliwowski, 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-I-1 and A-I-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-I_M 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 9. Distribution pattern of apoC-II and C-III in HDL of control Wistar rats, nondiabetic littermates, and SDBB rats^a

	C Apoproteins in HDL				
	C-II (pI 4.74)	C-III-0 (pI 4.67)	C-III-2(1) (pI 4.61)	C-III-3 (pI 4.5)	C-III-4 (pI 4.43)
	% of total				
Age-matched controls ^b (n = 5)	21.9 ± 1.5	48.2 ± 3.1	6.8 ± 0.8	20.9 ± 1.3	2.1 ± 0.3
Nondiabetic littermates ^b (n = 5)	31.0 ± 0.1 ^c	28.3 ± 1.9 ^c	6.7 ± 1.4	31.8 ± 2.2 ^c	2.0 ± 0.4
SDBB rats ^b (n = 8)	43.0 ± 2.9 ^c	23.8 ± 2.6 ^c	6.1 ± 0.6	25.2 ± 0.5 ^c	1.7 ± 0.1 ^d

^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 ($P < 0.05$).

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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REFERENCES

- Garcia, M. J., P. M. McNamara, T. Gordon, and W. B. Kannel. 1974. Morbidity and mortality in diabetics in the Framingham population. *Diabetes*. **23**: 105–111.
- Stout, R. W. 1979. Diabetes and atherosclerosis—the role of insulin. *Diabetologia*. **16**: 141–150.
- Lewis, B. 1976. The hyperlipidaemias. In *Clinical and Laboratory Practice*. Blackwell Scientific Publications, London. 294–303.
- Santen, R. J., P. W. Willis III, and S. S. Fajans. 1972. Atherosclerosis in diabetes mellitus. Correlation with serum lipid levels, adiposity, and serum insulin level. *Arch. Intern. Med.* **130**: 833–843.
- Oppl, J. J. 1980. Plasma lipoproteins in diabetes. In *CRC Handbook of Electrophoresis*. Vol. II—Lipoproteins in Disease. L. A. Lewis and J. J. Oppl, editors. CRC Press, Inc., Boca Raton, FL. 121–184.
- Wexler, B. C. 1970. Histopathologic responses to severe alloxan diabetes in atherosclerotic and nonatherosclerotic rats. *Diabetes*. **19**: 324–336.
- Wilson, R. B., J. M. Martin, and W. S. Hartroft. 1967. Evaluation of the relative pathogenic roles of diabetes and serum cholesterol levels in the development of cardiovascular lesions in rats. *Diabetes*. **16**: 71–82.
- Kalant, N., and W. A. Harland. 1961. The effect of an atherogenic diet on normal and alloxan-diabetic rats. *J. Can. Med. Assoc.* **84**: 251–254.
- Wellmann, K. F., and B. W. Volk. 1970. Experimental atherosclerosis in normal and subdiabetic rabbits. *Arch. Pathol.* **90**: 206–217.
- Bar-On, H., P. S. Roheim, and H. A. Eder. 1976. Serum lipoproteins and apolipoproteins in rats with streptozotocin-induced diabetes. *J. Clin. Invest.* **57**: 714–721.
- Chang, A. Y. 1978. Spontaneous diabetes in animals. *Gen. Pharmacol.* **9**: 447–450.
- Nakhooda, A. F., A. A. Like, C. I. Chappel, C. N. Wei, and E. B. Marliss. 1978. The spontaneously diabetic Wistar rat (the 'BB' rat). Studies prior to and during development of the overt syndrome. *Diabetologia*. **14**: 199–207.
- Marliss, E. B., A. A. F. Sima, and A. F. Nakhooda. 1981. Spontaneous diabetes in the 'BB' Wistar rat. In *Etiology and Pathogenesis of Insulin-dependent Diabetes Mellitus*. J. M. Martin, R. M. Ehrlich, and F. J. Holland, editors. Raven Press, New York. 251–274.
- Kalant, N., and T. Seemayer. 1979. Malignant lymphoma in spontaneously diabetic rats. *N. Engl. J. Med.* **300**: 737.
- Wright, J. R., Jr., A. J. Yates, N. T. Shah, J. C. Neff, D. W. Covey, and P. Thibert. 1980. Histological characteristics of the BB Wistar rat. *Vet. Clin. Pathol.* **12**: 9–13.
- Wright, J. R., Jr., A. J. Yates, H. M. Sharma, C. Shim, R. L. Tinger, and P. Thibert. 1982. Testicular atrophy in the spontaneously diabetic BB Wistar rat. *Am. J. Pathol.* **108**: 72–79.
- Wright, J. R., Jr., H. M. Sharma, A. J. Yates, B. M. Thompson, and P. Thibert. 1983. Supradiaphragmatic accessory lobe of the liver in BB Wistar rats. *Teratology*. **27**: 117–120.
- Wright, J. R., Jr., D. A. Senhauser, A. J. Yates, H. M. Sharma, and P. Thibert. 1983. Spontaneous thyroiditis in BB Wistar diabetic rats. *Vet. Pathol.* **20**: 522–530.
- Wright, J. R., Jr., A. J. Yates, H. M. Sharma, and P. Thibert. 1983. Central pontine myelinolysis following saline treatment of a diabetic rat for dehydration. *J. Comp. Pathol.* **93**: 509–514.
- Wright, J. R., Jr., A. J. Yates, H. M. Sharma, and P. Thibert. 1983. Pathological lesions in the spontaneously diabetic BB Wistar rat: a comprehensive autopsy study. *Metabolism*. **32(Suppl. 1)**: 101–105.
- Mendell, J. R., Z. Sahenk, J. R. Warmolts, J. K. Marshall, and P. Thibert. 1980. Peripheral nerve abnormalities in the spontaneously diabetic ('BB') Wistar rat. *Neurology*. **30**: 434.
- Subbiah, M. T. R., and D. Dietemeyer. 1980. Altered synthesis of prostaglandins in platelet and aorta from spontaneously diabetic Wistar rats. *Biochem. Med.* **23**: 231–235.
- Appel, M. C., A. A. Like, A. A. Rossini, D. B. Carp, and T. B. Miller. 1981. Hepatic carbohydrate metabolism in the spontaneously diabetic Bio-Breeding Worcester (BB/W) rat. *Am. J. Physiol.* **240**: E83–E87.
- Like, A. A., L. Butler, R. M. William, M. C. Appel, E. J. Weringer, and A. A. Rossini. 1982. Spontaneous autoimmune diabetes mellitus in the BB rat. *Diabetes*. **27(Suppl. 1)**: 7–13.
- Wright, J. R., Jr., A. J. Yates, H. M. Sharma, and P. Thibert. 1981. Spontaneous gastric erosions and ulceration in BB Wistar rats. *Lab. Anim. Sci.* **31**: 63–66.
- Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34**: 1345–1353.
- Koga, S., L. Bolis, and A. M. Scanu. 1971. Isolation and characterization of subunit polypeptides from apoproteins of rat serum lipoproteins. *Biochim. Biophys. Acta.* **236**: 416–430.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
- Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13**: 757–768.

30. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1969. Studies on the proteins in human plasma very low density lipoproteins. *J. Biol. Chem.* **244**: 5687-5694.
31. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solution of ethanol of the small molecular weight peptides of the serum very low density and high density lipoproteins. Relevance to the recovery problem during delipidation of serum lipoproteins. *Anal. Biochem.* **44**: 576-588.
32. Shore, V. G., R. E. Garcia, A. L. Penn, and B. Shore. 1980. Polyacrylamide gel electrophoresis and isoelectric focusing of plasma apolipoproteins. In *CRC Handbook of Electrophoresis*. Vol. 1. L. A. Lewis and J. J. Oppllt, editors. CRC Press, Boca Raton, FL. 103-125.
33. Weidman, S. W., B. Suarez, J. M. Falko, J. L. Witztum, J. Kolar, M. Raben, and G. Schonfeld. 1979. Type III hyperlipoproteinemia: development of a VLDL apoE gel isoelectric focusing technique and application in family studies. *J. Lab. Clin. Med.* **93**: 549-569.
34. Vesterberg, O., and L. Hansen. 1977. Staining of proteins in polyacrylamide gels. In *Electrofocusing and Isotachophoresis*. Proceedings of the International Symposium Aug. 2-4, 1976, Hamburg, Germany. B. J. Radolla and D. Graesslin, editors. Walter de Gruyter, Berlin. 124-133.
35. Catapano, A. L., R. L. Jackson, E. B. Gilliam, A. M. Gotto, Jr., and L. C. Smith. 1978. Quantification of apoC-II and apoC-III of human very low density lipoproteins by analytical isoelectric focusing. *J. Lipid Res.* **19**: 1047-1052.
36. Herbert, P. N., H. G. Windmueller, T. P. Bersot, and R. S. Shulman. 1974. Characterization of the rat plasma lipoproteins. I. The low molecular weight proteins of rat plasma high density lipoproteins. *J. Biol. Chem.* **249**: 5718-5724.
37. Gidez, L. I., J. B. Swaney, and S. Murnane. 1977. Analysis of rat serum apolipoproteins by isoelectric focusing. I. Studies on the middle molecular weight subunits. *J. Lipid Res.* **18**: 59-68.
38. Swaney, J. B. and L. I. Gidez. 1977. Analysis of rat serum apolipoproteins by isoelectric focusing. II. Studies on the low molecular weight subunits. *J. Lipid Res.* **18**: 69-76.
39. Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20**: 470-475.
40. Sampson, E. J., L. M. Demers, and A. F. Krieg. 1975. Faster enzymatic procedure for serum triglycerides. *Clin. Chem.* **21**: 1983-1985.
41. Newman, H. A. I., and R. W. Jenny. 1979. Enzymatic measurement of HDL cholesterol on fractions separated by electrophoresis, ultracentrifugation, or precipitation. In *Report on the High Density Lipoprotein Methodology Workshop*. K. Lippel, editor. NIH Publication No. 79-1661, U.S. Dept. of Health, Education and Welfare, Bethesda, MD. 99-106.
42. O'Brien, M., S. T. Patel, A. Mukhopadhyay, H. A. I. Newman, D. R. Feller, S. S. Kokrady, D. T. Witiak, R. R. Lanese, and J. C. Rice. 1981. Hypolipidemic effect of clofibrate and selected chroman analogs in fasted rats. I. Chow-fed animals. *Lipids*. **16**: 903-911.
43. Stavropoulos, W. S., and R. D. Crouch. 1974. A new colorimetric procedure for the determination of serum triglycerides. *Clin. Chem.* **20**: 857.
44. SAS Users Guide, 1979 ed. SAS Institute Inc., Raleigh, NC. 1979.
45. Kruskal, W. H., and W. A. Wallis. 1952. Use of ranks in one-criterion variance analysis. *J. Am. Stat. Assoc.* **47**: 583-621.
46. Connelly, P. W., and A. Kuksis. 1982. SDS-glycerol polyacrylamide gel electrophoresis of plasma apolipoproteins. *Biochim. Biophys. Acta.* **711**: 245-251.
47. Van Tol, A. 1977. Hypertriglyceridemia in the diabetic rat. Defective removal of serum very low density lipoproteins. *Atherosclerosis*. **26**: 117-128.
48. Weiland, D., C. E. Mondon, and G. M. Reaven. 1980. Evidence for multiple causality in the development of diabetic hypertriglyceridaemia. *Diabetologia*. **18**: 335-340.
49. Bar-On, H., and S. Eisenberg. 1978. The metabolic fate of high density lipoprotein (HDL) in the diabetic rat. *Diabetologia*. **14**: 65-69.
50. Nikkilä, E. A., and P. Hormila. 1978. Serum lipids and lipoproteins in insulin-treated diabetes. Demonstration of increased high density lipoprotein concentrations. *Diabetes*. **27**: 1078-1086.
51. Kennedy, A. L., T. R. J. Lappin, T. D. Lavery, D. R. Hadden, J. A. Weaver, and D. A. D. Montgomery. 1978. Relation of high density lipoprotein cholesterol concentration to type of diabetes and its control. *Br. Med. J.* **2**: 1191-1194.
52. Elkeles, R. S., J. Wu, and J. Hambley. 1978. Haemoglobin A1, blood glucose, and high density lipoprotein cholesterol in insulin-requiring diabetics. *Lancet*. **II**: 547-549.
53. Witztum, J. L., and G. Schonfeld. 1978. Carbohydrate diet-induced changes in very low density lipoprotein composition and structure. *Diabetes*. **27**: 1215-1229.
54. La Rosa, J. C., R. I. Levy, P. Herbert, S. E. Lux, and D. S. Fredrickson. 1970. A specific apoprotein activator for lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **41**: 57-62.
55. Havel, R. J., V. G. Shore, B. Shore, and D. M. Bier. 1970. Role of specific glycopeptides of human serum lipoproteins in the activation of lipoprotein lipase. *Circ. Res.* **27**: 595-600.
56. Ekman, R., and P. Nilsson-Ehle. 1975. Effects of apolipoproteins on lipoprotein lipase activity of human adipose tissue. *Clin. Chim. Acta.* **63**: 29-35.
57. Brown, W. V., and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem. Biophys. Res. Commun.* **46**: 375-381.
58. Gabor, J., M. Spain, and N. Kalant. 1980. Composition of serum very-low-density and high-density lipoproteins in diabetes. *Clin. Chem.* **26**: 1261-1265.
59. Dodson, P. M., J. Stocks, G. Holdsworth, and D. J. Galton. 1980. The apolipoprotein C composition of triglyceride-rich lipoproteins of patients with chronic renal failure: the effect of dialysis. *J. Clin. Chem. Clin. Biochem.* **18**: 751-752.
60. Stocks, J., G. Holdsworth, P. Dodson, and D. J. Galton. 1981. An abnormal triglyceride-rich lipoprotein carrying excess apolipoprotein C-II. *Atherosclerosis*. **38**: 1-9.
61. Weisweiler, P., D. Jungst, and P. Schwandt. 1983. Quantitation of apolipoprotein E-isoforms in diabetes mellitus. *Horm. Metab. Res.* **15**: 201.
62. Sliwowski, M. B., A-L. Wu, and H. G. Windmueller. 1983. Metabolic interconversion of apolipoprotein A-I isoforms in the rat. *Federation Proc.* **42**: 1817.
63. Zannis, V. I., J. L. Breslow, and A. J. Katz. 1980. Isoforms of human apolipoprotein A-I demonstrated in

- plasma and intestinal organ culture. *J. Biol. Chem.* **255**: 8612-8617.
64. Zannis, V. I., A. M. Lees, R. S. Lees, and J. L. Breslow. 1982. Abnormal apoprotein A-I isoprotein composition in patients with Tangier disease. *J. Biol. Chem.* **257**: 4978-4986.
65. Zannis, V. I., D. M. Kurnit, and J. L. Breslow. 1982. Hepatic apoA-I and apoE and intestinal apoA-I are synthesized in precursor isoprotein forms by organ culture of human fetal tissues. *J. Biol. Chem.* **257**: 536-544.
66. Franceschini, G., C. R. Sirtori, A. Capurso, K. H. Weisgraber, and R. W. Mahley. 1980. Decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. *J. Clin. Invest.* **66**: 892-900.
67. Weisgraber, K. H., T. P. Bersot, R. W. Mahley, G. Franceschini, and C. R. Sirtori. 1980. A-I^{Milano} apoprotein: isolation and characterization of a cysteine-containing variant of A-I apoprotein from human high density lipoproteins. *J. Clin. Invest.* **66**: 901-907.
68. Kay, L. L., R. Ronan, E. J. Schaefer, and H. B. Brewer, Jr. 1982. Tangier disease: a structural defect in apolipoprotein A-I (apoA-I^{Tangier}). *Proc. Natl. Acad. Sci. USA.* **79**: 2485-2489.
69. Franceschini, G., M. Sirtori, G. Gianfranceschi, and C. R. Sirtori. 1981. Relation between the HDL apoproteins and A-I isoproteins in subjects with the A-I^{Milano} abnormality. *Metabolism.* **30**: 502-509.
70. Rosseneu, M., G. Assmann, M. J. Taveirne, and G. Schmitz. 1984. Lipid binding properties of the Tangier apolipoprotein A-I and its isoproteins. *J. Lipid Res.* **25**: 111-120.