Characterization of the plasma lipoproteins of the genetically obese hyperlipoproteinemic Zucker fatty rat

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Abstract The plasma lipoproteins of the Zucker fatty rat were characterized with respect to lipid and apoprotein composition, and results were compared with those obtained from lean controls. Information on apoproteins was obtained from gel filtration experiments and electrophoresis on polyacrylamide gels. Very low density lipoproteins (VLDL) were increased severalfold in fatties, and 78% of their mass was triglycerides compared with 60% in the controls. Low density (LDL) and high density (HDL) lipoproteins were increased by a factor of 2, although their compositions were similar to those of the controls. Levels of apoVLDL, apoLDL, and apoHDL were five, two, and two times higher, respectively, in the fatties, and the two most rapidly moving subunit peptides on polyacrylamide gels were disproportionately elevated in the apoproteins. The slower of these two bands was present in relatively greater amounts than the faster one in fatties. If the slower peptide is an activator of lipoprotein lipase, analogous to the comparable subunit peptides of human apolipoproteins, plasmas of fatties could contain up to 10 times more lipase activator activity than control plasma. This finding, and the fact that adipose tissue lipoprotein lipase activity of fatties was about 150% of controls, suggests that fatties have increased capacities for VLDL catabolism. We have previously shown that hepatic VLDL secretory rates are higher than normal in these animals. The increased capacity for catabolism may be a response to the altered secretory rates.

Supplementary key words apolipoproteins · lipoprotein lipase

The Zucker fatty rat (1) is genetically obese. Its adipose tissue, which makes up over 40% of total body weight, contains abnormally large numbers of large fat cells, which synthesize fatty acids and glycerides at increased rates (2–4). Both adipose tissue and muscle may be insulin resistant as well (5–7). The fatty rat is also hyperlipoproteinemic (8–10). In order to understand the mechanisms responsible for the hyperlipoproteinemia, it was important first to characterize the plasma lipoproteins of these animals. Therefore, lipoproteins isolated from plasma of individual animals were analyzed. Particular attention was paid to the apolipoproteins because they are important in lipoprotein synthesis, structural stability, and catabolism (11–14). Adipose tissue lipoprotein lipase, an enzyme that participates in lipoprotein catabolism (15), was also studied. To place these studies in a somewhat broader setting, the results of blood lipid analyses were also compared with a more frequently studied strain of rat, the Sprague-Dawley.

Abbreviations: S-D, Sprague-Dawley; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; chol, cholesterol; PL, phospholipid; FFA, free fatty acid; IRI, immunoreactive insulin; SDS, sodium decyl sulfate; PAGE, polyacrylamide gel electrophoresis.

METHODS

Isolation of lipoproteins

Fatty rats and normal weight (lean) controls, purchased from Dr. Lois Zucker (Stowe, Mass.), and Sprague-Dawley rats (S-D) were fed Purina rat chow and tap water ad lib. Food and water were removed at 8 a.m. Between 2 and 3 p.m., animals were anesthetized with pentobarbital (5 mg/100 g, intraperitoneally), and blood was collected from the aorta into tubes containing disodium EDTA, 1 mg/ml. Plasmas were separated promptly, and isolation of lipoproteins was begun on the same day. Lipoproteins were isolated from a number of individual animals. Separate pools of plasma were also made using fatty and control plasmas. 4 ml of plasma was overlaid with EDTA–saline (0.16 M NaCl, 1 mM in disodium EDTA, pH 8.6) and centrifuged at 40,000 rpm for 16 hr (or 1×10^8 *g*-min) at 10°C in a 40.3 rotor in a Beckman model L2-65B preparative ultracentrifuge (16). The top 2 ml, obtained by slicing the tube, was overlaid with EDTA–saline and recentrifuged as above. This fraction contained very low density lipoprotein (VLDL). To obtain low density lipoprotein (LDL) and high density lipoprotein (HDL), the density of the infranate of the first VLDL ul-

tracentrifugation was raised to a calculated density of 1.21 with solid KBr, and the fraction was centrifuged in a Ti-60 rotor at 50,000 rpm for 20–24 hr (or 2×10^8 *g*-min). The top 3 ml of this fraction, again obtained by slicing the tube, was overlaid with a KBr solution of density 1.21 (1 mM in disodium EDTA, pH 8.6) and recentrifuged. The supernate of the second spin at d 1.21, which contained HDL and LDL, was diluted to a calculated density of 1.063 with EDTA–saline and centrifuged as above in the Ti-60 rotor for 18 hr. The top 2 ml of the centrifugation at d 1.063 was called LDL, and the infranate, HDL. To obtain a better approximation of the density distribution of the lipoproteins, pools of plasma obtained from four or five animals were subjected to ultracentrifugation at "narrower" densities. The following fractions were isolated: $S_f > 400$, S_f 20–400, d 1.006–1.018, d 1.018–1.050, d 1.050–1.070, and d 1.070–1.21. The first two fractions were isolated in a SW-40 rotor at 3×10^6 *g*-min and 1×10^8 *g*-min, respectively. The remaining fractions were isolated in a Ti-60 rotor at 2×10^8 *g*-min. Densities of salt solutions, prepared in parallel with the plasma fractions, were verified by pycnometry. The lipoproteins isolated above were subjected to agarose electrophoresis (17). Plates were stained for lipid with Fat Red 7B and for protein with Amido Schwartz. All lines that stained for protein also stained for lipid. Immunoelectrophoresis (18) was also carried out using an anti-rat serum (Boehringer). Single arcs in the appropriate locations were obtained. Slides were stained with Fat Red 7B. All arcs stained for lipid. Thus, there appeared to be no "contamination" by nonlipoprotein proteins.

Chemical determinations

Triglycerides (TG) and cholesterol (chol) were measured by the AutoAnalyzer II techniques in the Core Laboratory of the Lipid Research Center (19). Phospholipids (PL) were assessed by the methods of Bartlett (20) on extracts prepared according to Carlson (21). Plasma FFA was extracted by the method of Dole (22) and quantified colorimetrically according to Duncombe (23). Protein was determined by the method of Lowry et al. (24), using bovine serum albumin standards (Sigma, St. Louis). Reaction tubes were cleared by extraction with diethyl ether before determination of absorbance (25). 80–85% of the total plasma cholesterol and phospholipids and 75–80% of the triglycerides were recovered in the three density fractions. Recoveries of lipids from the pooled fractions were 70–75%. The results reported below have not been corrected for these losses. Plasma insulin (IRI) was measured by a double-antibody radioimmunoassay (26). We are grateful to Dr. G. T. Peake for these measurements.

Quantification of apoproteins

Apolipoprotein compositions were determined by gel filtration and by disc electrophoresis. Gel filtration was performed on VLDL and HDL. VLDL was extracted according to Brown, Levy, and Fredrickson (27) and was solubilized in solutions of 0.05 M $NaHCO_3$, pH 8.6, 0.1 M in sodium decyl sulfate (SDS). The apoprotein solutions (1–2 mg of protein) were filtered on a 0.9×60 cm column of Sephadex G-150 equilibrated with 0.05 M $NaHCO_3$, pH 8.6, 0.002 M in SDS. HDL was delipidated in a biphasic system with ether–ethanol solutions (28). ApoHDL was chromatographed on 2.5×90 cm columns packed with Sephadex G-200 with 0.1 M Tris, pH 8.0, 8 M in urea (11). Column chromatographic fractions were monitored at 280 nm, and the areas under the peaks were quantified by planimetry. The relative areas of duplicate samples processed in this manner agreed to within 10% of each other. Recoveries of apoproteins from the columns, as judged by protein analyses (24), were 80–85%.

Apolipoproteins were obtained for disc electrophoresis (29) by delipidation of lipoproteins. Apolipoproteins were then solubilized and applied to the gel columns. VLDL and LDL were delipidated and solubilized according to Brown et al. (27) as described above. Solubilized apoVLDL and apoLDL were diluted 1:5 to 1:10 with 0.1 M Tris, pH 8.6, 8 M in urea, before electrophoresis. HDL was delipidated according to Scanu and Edelstein (28). ApoHDL was solubilized in 0.1 M Tris, pH 8.6, 8 M in urea. 6-inch electrophoretic tubes containing 9% polyacrylamide gels, pH 8.9, 8 M in urea, and a Canalco apparatus (Rockville, Md.) were used. At the end of electrophoresis, the gels were fixed in 10% trichloroacetic acid, stained with Coomassie blue, destained in 7% acetic acid (30), and scanned at 550 nm with a Gilford model 2400-S spectrophotometer. The areas under the curves produced by each of the electrophoretic bands were quantified by planimetry. Areas were summed and the contributions of individual apoproteins or of groups of apoproteins were determined as percentages of the total area. Samples run in duplicate on the same day agreed to within 8% of each other. Samples run on different days agreed to within 12%. 5-, 10-, and 15-μg samples of apoHDL were subjected to electrophoresis to verify the linearity of absorbance of the stained discs with increasing protein loads. The percentage compositions of individual apoproteins varied by less than 10%; 15 μg of protein was applied routinely.

Enzyme assays

Lipoprotein lipase of epididymal fat pads was assayed according to Shotz et al. (31). [^{14}C]Triolein-containing substrates and extracts of adipose tissue powders were prepared at room temperature as follows.[1] About 5 g of

[1] We are grateful to Dr. J. Williams of Dr. P. R. Vagelos's laboratory for teaching us this method.

TABLE 1. Plasma lipid and insulin levels in fatty and control rats

	Body Weight	TG	Chol	PL	FFA	IRI
	g		*mg/dl*		*μeq/l*	*μU/ml*
Individual plasmas						
Fatty (13)[a]	514 ±61	1045 ±883	182 ±55	355 ±103	1069 ±470	89 ±21
Controls (12)	334 ±88	82 ±41	76 ±13	165 ±30	433 ±75	33 ±13
S-D (21)	272 ±22	74 ±25	66 ±7	107 ±13	473 ±74	26 ±15
Pooled plasmas						
Fatty (4)	347 ±41	233	249	248		
Controls (5)	212 ±29	39	67	56		

Total TG, chol, PL, FFA, and IRI were determined on plasmas of individual animals. Lipids were also determined on plasma pools prepared from a different set of animals. Values are means ± SD.

[a] The number of animals examined individually or contributing to each pool is in parentheses.

fat was removed and was rinsed free of blood, blotted, and homogenized in a VirTis apparatus in 10 vol of *n*-butanol. The homogenate was filtered under suction, and the residue was washed with 20–30 vol of petroleum ether. Powders were dried in vacuum desiccators at room temperature for 24 hr and then stored dry at −20°C. The tissue powders were extracted twice with 5 mM Tris at 4°C for 60 min. Two extractions were used because only 70–80% of the total extractable activity was recovered with one extraction. A third extraction yielded no detectable activity. The solutions were then centrifuged at 4°C for 20 min at 1680 *g*, and the supernatant solution was used immediately. Enzymatic activity in the lipase assays was linear over 40 min, and activity was directly related to protein concentration over a threefold range. Results are expressed in nanomoles of oleic acid released per minute from the triolein substrates per milligram of extracted protein. Duplicate incubations agreed to within 5% of the mean, and assays run on different days agreed within 9% (coefficient of variation).

RESULTS

Fatties weighed considerably more than lean controls of identical age and had plasma levels of lipids, including FFA, that were 2–20 times normal (**Table 1**). Pooled plasma, collected from younger animals, had lower levels of lipids, but the differences between the fatty and control pools were equally dramatic. Lean controls and S-D rats had comparable lipid levels. Plasma IRI was increased in fatties compared with control and S-D rats.

VLDL levels were about seven times higher and LDL and HDL concentrations were each about two times higher in fatties than in controls (**Table 2**). However, in fatties, individual differences in VLDL (and TG) levels were great. HDL made up about 62% of the plasma lipoprotein mass in controls, whereas VLDL was clearly the predominant species in fatties, constituting 52% of the total lipoprotein mass.

A more detailed breakdown of the density distribution of lipoproteins was obtained from two plasma pools, one prepared from fatty and the other from control animals. In these plasma pools, the triglyceride-rich lipoproteins,

TABLE 2. Lipoprotein levels in fatty and control rats

	VLDL	LDL	HDL
		mg/dl	
Individual rats			
Fatty (4)	623 ±125	105 ±2	468 ±57
Control (4)	87 ±27	62 ±13	241 ±23

	$S_f > 400$	S_f 20–400	d 1.006–1.018	d 1.018–1.050	d 1.050–1.070	d 1.070–1.21
			mg/dl			
Pooled plasmas						
Fatty (4)	110	130	2.2	98	16	442
	14[a]	*16*	*0.2*	*12*	*2*	*55*
Control (5)	15	27	1.7	16	5	123
	8	*14*	*0.9*	*9*	*3*	*65*

Lipoproteins were isolated from four fatties and four controls (VLDL = d < 1.006, LDL = d 1.006–1.063, HDL = d 1.063–1.21). Values are means ± SD. Pools were composed of plasmas from four fatties and five controls; results represent total TG, Chol, PL, and protein (by weight) in each lipoprotein fraction. The mass contributed by fatty acids in cholesteryl esters was ignored.

[a] Values in italics are percentages of total lipoproteins, e.g., mass of $S_f > 400 \times 100$/ sum of all density fractions.

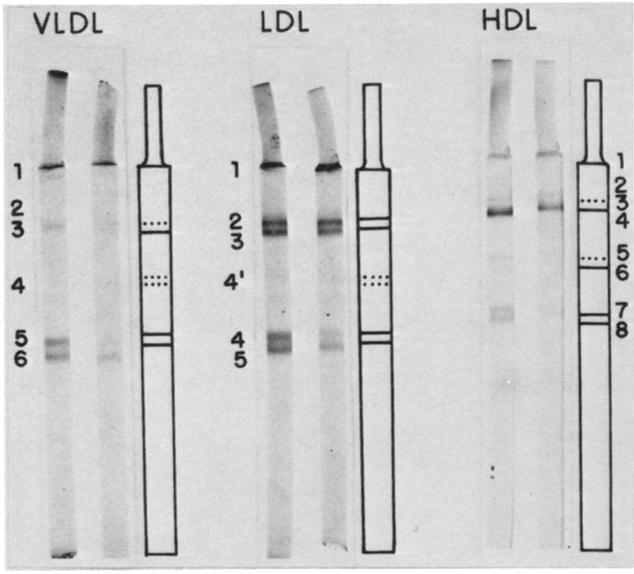

Fig. 1. Apolipoproteins of fatty and lean control rats separated in 9% polyacrylamide, 8 M in urea, pH 8.9; Coomassie blue stain. Nomenclature employed is that of Koga et al. (11). The left-hand member of each pair of disc gels represents the fatty, the right-hand member, the control.

S_f > 400 and S_f 20–400, were present in approximately equal amounts, together making up 30% of the lipoprotein mass. The "intermediate" densities, d 1.006–1.018 and 1.050–1.070 each contained less than 5% of the lipoproteins. Thus, the lipemia in fatties consisted of increases in all of the density fractions that were present in the controls, without the generation of any lipoproteins of "intermediate" densities.

Mean VLDL TG contents (TG as percentage of VLDL mass) were 78 ± 14% and 64 ± 8% in fatties and controls, respectively. The percentages of VLDL TG in controls and S-D rats were similar. Chol, PL, and protein, respectively, made up >2%, 14 ± 2%, and 6 ± 1% of VLDL mass in fatties and 10 ± 2%, 16 ± 5%, and 9 ± 2% in controls. The compositions of the fatty and control S_f > 400 and S_f 20–400 pools were similar. The fatty and control S_f > 400 fractions consisted of 76 and 74% TG, and the S_f 20–400 fractions contained 70 and 69% TG in fatties and controls, respectively. Thus, the enrichment of the VLDL by TG in fatties was probably due to the greater proportion of S_f > 400 in fatty VLDL rather than to differences in the compositions of comparable density fractions.

Gross compositions of LDL and HDL in fatties were as follows: TG, chol, PL, and protein, <2%, 26 ± 4%, 52 ± 8%, and 20 ± 5% in LDL and <2%, 17 ± 2%, 42 ± 5%, and 41 ± 4% in HDL. These values did not differ from those of controls. These values were somewhat higher for phospholipids and lower for cholesterol than the results reported for other strains (13). The differences probably reflect the presence of some HDL in the 1.006–1.063 fractions, because the compositions of the 1.018–1.050 pooled plasma fractions were closer to those reported by others (13): TG, chol, PL, and protein were 1, 44, 32, and 23%, respectively. These latter results were obtained from the pooled plasma of fatties. The compositions of the comparable control and fatty density fractions were not significantly different.

The apolipoproteins of the fatty and control groups resembled those described in other rats (11–13, 32). Representative samples of the polyacrylamide gels used for quantification of the apoproteins are shown in **Fig. 1.** Since the nomenclature of these proteins has not been standardized (11, 12, 32), we followed the nomenclature of Koga, Horwitz, and Scanu (11) (Fig. 1). There are pitfalls in this procedure because the bands designated by the PAGE numbers may not represent the same protein designated by Koga et al. (11). Nevertheless, the procedure has the merit of not introducing still another nomenclature. It is reasonably certain that bands 5 and 6 of VLDL, 4 and 5 of LDL, and 7 and 8 of HDL correspond to each other (11). Bands 1 in VLDL and LDL are also probably the same. It is not clear how bands 2, 3, and 4 of VLDL are related to bands 3, 4, 5, and 6 of HDL. In apoLDL we found a band not described by Koga et al. (11) that corresponded to band 4 of apoVLDL. In order to keep the nomenclature comparable to that of Koga et al., we have called this band apoLDL 4′.

ApoVLDL was fractionated by column chromatography into effluent peaks I and II (**Fig. 2**). Others have obtained three peaks using more protein and longer columns (11, 12). We used a short column because the amounts of VLDL protein available are limited. Peak II contained primarily bands 5 and 6 by PAGE and a hazy area near band 4. Peak I contained band 1 and small amounts of bands 2, 3, and 4. The separation obtained with the short

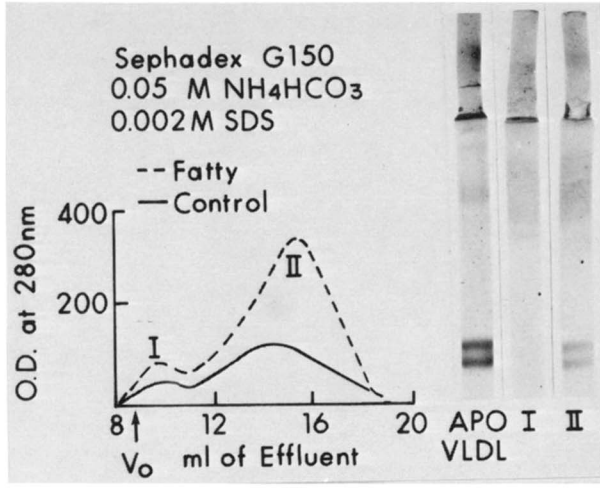

Fig. 2. Gel filtration chromatography of rat apoVLDL on a 0.9 × 45 cm column of Sephadex G-150. Peaks were pooled and analyzed by PAGE. Disc gels are those of the fatty rat. Peak I contained bands 1, 2, 3, and 4. Peak II contained bands 5 and 6 and a small amount of band 4. Fatty total TG = 729 mg/dl; lean total TG = 119 mg/dl.

TABLE 3. Apolipoprotein composition of the rat lipoproteins

	VLDL			LDL			HDL		
Band	2 + 3	4	5 + 6	2 + 3	4′	4 + 5	3 + 4	5 + 6	7 + 8
Fatties (12)	25[a] ±6	<2	75[a] ±7	43[a] ±9	9 ±8	49[a] ±6	61[a] ±6	14 ±5	24[a] ±8
Controls (9)	34 ±9	<2	59 ±13	61 ±2	5 ±1	36 ±2	70 ±9	16 ±6	17 ±4

Lipoproteins were isolated, delipidated, solubilized, and separated by disc electrophoresis in 9% polyacrylamide, 8 M in urea, pH 8.9. Gels were stained with Coomassie blue and proteins were quantified by planimetry after scanning at 550 nm in a Gilford 2400-S spectrophotometer. Results are expressed as percentages of total area scanned (means ± SD). The apoprotein nomenclature is that of Koga et al. (11) (see Fig. 1). The results represent the relative proportions of those proteins that penetrated the gels. The number of animals in each group is in parentheses.

[a] Difference from control significant at $P < 0.02$.

column is comparable to that achieved with the longer ones except for bands 2, 3, and 4, which usually elute in the "middle" peak that we did not obtain. However, this does not invalidate data relevant to peak II. Peak II represented 77 and 89% of apoVLDL in control and fatty rats, respectively. When apoVLDL was analyzed by PAGE, its band "5 + 6" content was increased relative to band "2 + 3" (5 + 6/2 + 3 = 3/1 for fatties compared with 1.7/1 for controls, **Table 3**). Thus, in fatties the amounts of fast-moving bands 5 and 6 were disproportionately high with respect to apoVLDL. The band 4 + 5 content of LDL relative to band 2 + 3 was also increased.

The component proteins of four apoHDL preparations, two fatty and two control, were analyzed by column chromatography and by PAGE (**Fig. 3**). Three chromatographic elution peaks (I, II, and III) were obtained, corresponding to PI, PIII-IV, and PV of Koga et al. (11); by analytical PAGE, our peaks II and III were composed of bands 3 and 4 and bands 5, 6, 7, and 8, respectively. The chromatographic elution peaks were quantified by planimetry. Peak II contained 60–65% and peak III 15–30% of the apoHDL recovered from the column; total recovery was 80–85%. ApoHDL was also analyzed by PAGE. Bands 3 and 4 contained 60–70% and bands 7 and 8 16–27% of the total area. Thus, the chromatographic and PAGE procedures agreed reasonably well. Peak III contained 25 and 30% of the total protein in the two fatty apoHDL preparations. The analogous results for the controls were 15 and 20%. By PAGE, bands 7 and 8 contained 24 and 27% of apoHDL in fatties and 16 and 17% in controls.

Based on the above results, the approximate levels of bands 5 and 6 associated with VLDL were 32 and 4.4 mg/dl and the levels of bands 7 and 8 associated with HDL were 41 and 19 mg/dl in fatties and controls, respectively. These are, of course, rough estimates because the chromogenicity of the various bands is not known.

Fast-moving bands (apoVLDL 5 and 6, apoLDL 4 and 5, and apoHDL 7 and 8) were found in each of the "narrow" density fractions analyzed by PAGE. The relative proportions of these bands (5, 4, or 7:6, 5, or 8, respectively) as quantified by PAGE were about 35:65 for controls and 45:55 for fatties. These differences were significant at $P < 0.01$. In any given animal, the proportions of these bands to each other in VLDL, LDL, and HDL were similar. Unique or novel apoproteins were not detected in the lipoproteins of fatty rats compared with controls, nor were any of the apoproteins deleted.

Lipoprotein lipase activity of epididymal adipose tissue was determined on extracts of tissue powders prepared with *n*-butanol–petroleum ether. The enzyme activity of the adipose tissue of fatties (1.35 ± 0.28 nmoles of oleate/mg of protein/min, mean ± SD, n = 6) exceeded that of controls (0.92 ± 0.29, n = 8, $P < 0.02$). The activity of the latter was similar to that found in S-D rats (0.80 ± 0.29, n = 4). More than 90% of the enzyme activity of these preparations was inhibited when incubations were carried out in 1 M NaCl or when enzyme preparations were preincubated in 1 M NaCl at 4°C for 30 min prior to assay.

DISCUSSION

Plasma levels of all lipids including FFA were increased in fatties. Hyperinsulinemia was present as well. Lean controls, on the other hand, had plasma lipid and IRI levels closely resembling those of the S-D strain. These findings confirm and extend previous observations (6–10).

The division of the lipoproteins into the density classes initially chosen for isolation was arbitrary because analytical ultracentrifugal data on this strain of rat are not available. However, the results obtained from the plasma pools indicate that the distribution of the density classes is not unlike those of other strains. Rat LDL has been isolated at densities ranging from lower densities of 1.006 to 1.019 to higher densities of 1.035 to 1.063. Although most workers agree that the mean density of rat LDL is somewhat lower than that of human LDL (11, 12, 32), densities are affected by diet (33). Most of the LDL in these animals seems to float between d 1.018 and d 1.050 (Table

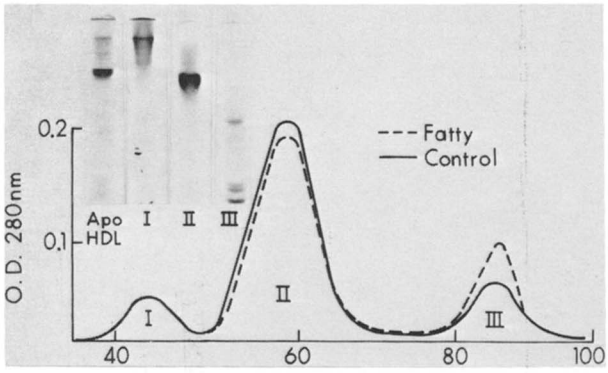

Fig. 3. Gel filtration chromatography of rat apoHDL on a 2.5 × 90 cm column of Sephadex G-200 equilibrated with 0.1 M Tris, pH 8, 8 M in urea. Disc gels are those of control rats. Peak I contained bands 1 and 2; peak II, bands 3 and 4; and peak III, bands 5, 6, 7, and 8.

2). Nevertheless, because we wished to include all lipoproteins and apoproteins in the comparison of fatties with controls, we chose to isolate LDL in the more inclusive density range of 1.006–1.063, even though this entailed the risk of some contamination of LDL by other density fractions.

It is clear from the individual and the pool data that in fatties all lipoprotein density classes are increased. The major elevation is in VLDL, which in fatties on the average was increased about sixfold and represented 30–52% of the total lipoprotein mass vs. 22–23% in controls. The hypertriglyceridemia was related to the accumulation of both $S_f > 400$ and S_f 20–400 particles in plasma (8); in the plasma pool, fatty $S_f > 400$ levels nearly equaled levels of S_f 20–400.

Fatty VLDL was enriched in triglyceride, the amount of enhancement being directly related to the level of VLDL triglyceride present. This probably represents the accumulation of larger TG-enriched particles in plasma. By withholding food between 8 a.m. and 2–3 p.m., we hoped to minimize the contribution of dietary fat to the circulating lipoproteins. However, there is no way of quantifying the relative contributions of gut mucosal and hepatic cells to the plasma lipoproteins of these animals. Thus, the partition of particles into $S_f > 400$ and S_f 20–400 classes carries no physiological implications, and from the compositional data alone no unequivocal conclusions may be drawn as to the relative roles of gut and liver in the hyperlipoproteinemia. Nevertheless, the findings are subject to some interpretations.

Accumulations of triglyceride-enriched, larger VLDL particles have been described in "carbohydrate-induced" hyperlipoproteinemia in man (34, 35). Obesity (10, 36), carbohydrate induction (37), and renal disease (38) (which fatties develop late in life) are associated with increased hepatic VLDL secretion rates, which in turn result in the secretion of larger (39), less dense (40) VLDL particles. Thus, the accumulation of large VLDL particles in fatty plasma probably reflects increased hepatic secretion, at least in part. The stimulus for the hypersecretion is not known, but it may be related to a number of factors such as food intake, glucose–insulin metabolism, or renal disease.

Although LDL levels of fatties were increased by a factor of 2, gross composition did not differ significantly from controls, nor was there a proportionate increase in the d 1.006–1.018 lipoproteins. Current concepts (41) hold that, at least in man, VLDL is catabolized in plasma to LDL, the latter being a catabolic product that is not synthesized de novo. According to this concept, human type III and IV hyperlipoproteinemias may represent defects in VLDL catabolism that result in accumulations of β- and pre-β-VLDL, respectively. A "catabolic block" analogous to human type IV cannot be ruled out in the fatty by our data, but the absence of cholesterol-enriched VLDL or LDL in fatties makes a "block" resembling type III unlikely.

In spite of the doubling in plasma levels, gross composition of fatty HDL did not differ from controls. Hypoalphalipoproteinemia in man may be associated with hypertriglyceridemia (42), presumably due to a deficiency of an activator protein for lecithin:cholesterol acyltransferase (43). The finding of normal HDL compositions and of more than adequate apoA levels in fatties rules out this mechanism as a factor in the hypertriglyceridemia of the fatty.

Among the most striking differences between fatties and lean controls were the increased fast-migrating band 5 and 6 peptide levels in all density fractions and the relatively greater abundance of band 5. Bands 5 and 6 appear to be synthesized only in liver (32) and may be secreted along with either VLDL or HDL or both. The high levels of 5 and 6 in fatty plasma, therefore, probably reflect increased secretion of VLDL (and perhaps of HDL) from the liver. The mechanism(s) responsible for the relative increase of band 5 relative to 6 is unknown. However, similar observations have been made in the carbohydrate-fed rat.[2] The apoproteins analogous to bands 5 and 6 in rat are probably the apoC group in man (44). Alterations in the composition of the apoC proteins in man occur with carbohydrate feeding[3] but not after an oral fat load (45, 46). Thus, it is possible that the rates of synthesis of these proteins relative to each other may be altered as the rates of secretion of VLDL are increased.

The accumulation of VLDL in plasma suggests an imbalance between input and output rates. The hyperlipemia of these animals persists even on calorie-restricted diets (9), when input is presumably decreased, suggesting that some defect in plasma clearance may be present. Kinetic data are needed to settle this point. However, to the extent

[2] Roheim, P. S. Personal communication.
[3] Schonfeld, G. Unpublished observations.

that such defects may be present, it is clear that an inability to synthesize lipoprotein lipase is not responsible. Indeed, if bands 5 and 6 are modulators of lipoprotein lipase activity (44), the changes in the ratio of band 5 to band 6 in fatties would tend to favor activation rather than inhibition of lipoprotein lipase activity (47, 48). It may be that these alterations in lipase and activator levels represent attempts at adaptation to increased VLDL secretion rates, which are insufficient to compensate for the greatly increased input. The fatty strain of rat, therefore, represents an interesting model of hyperlipoproteinemia. Further studies of lipoprotein synthesis and clearance from plasma should add considerably to knowledge of lipoprotein metabolism.

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REFERENCES

1. Zucker, L. M., and T. F. Zucker. 1961. Fatty, a new mutation in the rat. *J. Hered.* **52:** 275–278.
2. Zucker, T. F., and L. M. Zucker. 1963. Fat accretion and growth in the rat. *J. Nutr.* **80:** 6–19.
3. Bray, G. A. 1968. Lipogenesis from glucose and pyruvate in fat cells from genetically obese rats. *J. Lipid Res.* **9:** 681–686.
4. Johnson, P. R., L. M. Zucker, J. A. F. Cruce, and J. Hirsch. 1971. Cellularity of adipose depots in the genetically obese Zucker rat. *J. Lipid Res.* **12:** 706–714.
5. Stern, J., P. R. Johnson, M. R. C. Greenwood, L. M. Zucker, and J. Hirsch. 1972. Insulin resistance and pancreatic insulin release in the genetically obese Zucker rat. *Proc. Soc. Exp. Biol. Med.* **139:** 66–69.
6. Zucker, L. M., and H. N. Antoniades. 1972. Insulin and obesity in the Zucker genetically obese rat "fatty." *Endocrinology.* **90:** 1320–1330.
7. York, D. A., J. Steinke, and G. A. Bray. 1972. Hyperinsulinemia and insulin resistance in genetically obese rats. *Metabolism.* **21:** 277–284.
8. Zucker, L. M. 1965. Hereditary obesity in the rat associated with hyperlipemia. *Ann. N.Y. Acad. Sci.* **131:** 447–458.
9. Barry, W. S., and G. A. Bray. 1969. Plasma triglycerides in genetically obese rats. *Metabolism.* **18:** 833–839.
10. Schonfeld, G., and B. Pfleger. 1971. Overproduction of very low-density lipoproteins by livers of genetically obese rats. *Amer. J. Physiol.* **220:** 1178–1181.
11. Koga, S., D. L. Horwitz, and A. M. Scanu. 1969. Isolation and properties of lipoproteins from normal rat serum. *J. Lipid Res.* **10:** 577–588.
12. Bersot, T. P., W. V. Brown, R. I. Levy, H. G. Windmueller, D. S. Fredrickson, and V. S. LeQuire. 1970. Further characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry.* **9:** 3427–3433.
13. Camejo, G. 1967. Structural studies of rat plasma lipoproteins. *Biochemistry.* **6:** 3228–3241.
14. Fredrickson, D. S., A. M. Gotto, and R. I. Levy. 1972. Familial lipoprotein deficiencies. *In* Metabolic Basis of Inherited Diseases. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw Hill, New York. 493–530.
15. Robinson, D. S. 1963. The clearing factor lipase and its action in the transport of fatty acids between the blood and the tissues. *Advan. Lipid Res.* **1:** 133–182.
16. Hatch, F. T., and R. S. Lees. 1968. Practical methods for plasma lipoprotein analysis. *Advan. Lipid Res.* **6:** 1–68.
17. Noble, R. P. 1968. Electrophoretic separation of plasma lipoproteins in agarose gel. *J. Lipid Res.* **9:** 693–700.
18. Ouchterlony, O. 1968. Handbook of Immunodiffusion and Immunoelectrophoresis. Ann Arbor Science Publishers, Ann Arbor, Mich.
19. Manual of Laboratory Methods. 1972. Lipid Research Clinics Program. University of North Carolina, Chapel Hill.
20. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
21. Carlson, L. A. 1963. Determination of serum triglycerides. *J. Atheroscler. Res.* **3:** 334–336.
22. Dole, V. P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35:** 150–14.
23. Duncombe, W. G. 1963. The colorimetric micro-determination of long-chain fatty acids. *Biochem. J.* **88:** 7–10.
24. 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.
25. Gustafson, A., P. Alaupovic, and R. H. Furman. 1965. Studies of the composition and structure of serum lipoproteins: isolation, purification, and characterization of very low density lipoproteins of human serum. *Biochemistry.* **4:** 596–605.
26. Morgan, C. R., and A. Lazarow. 1963. Immunoassay of insulin: two antibody system. Plasma insulin levels of normal, subdiabetic and diabetic rats. *Diabetes.* **12:** 115–126.
27. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1970. Further characterization of apolipoproteins from the human plasma very low density lipoproteins. *J. Biol. Chem.* **245:** 6588–6594.
28. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solutions 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.
29. Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121:** 404–427.
30. Chrambach, A., R. A. Reisfeld, M. Wyckoff, and J. Zaccari. 1967. A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis. *Anal. Biochem.* **20:** 150–154.
31. Schotz, M. C., A. S. Garfinkel, R. J. Huebotter, and J. E. Stewart. 1970. A rapid assay for lipoprotein lipase. *J. Lipid Res.* **11:** 68–69.
32. Windmueller, H. G., P. N. Herbert, and R. I. Levy. 1973. Biosynthesis of lymph and plasma lipoprotein apoproteins by isolated perfused rat liver and intestine. *J. Lipid Res.* **14:** 215–223.
33. Lasser, N. L., P. S. Roheim, D. Edelstein, and H. A. Eder. 1973. Serum lipoproteins of normal and cholesterol-fed rats. *J. Lipid Res.* **14:** 1–8.
34. Schonfeld, G. 1970. Changes in the composition of very low density lipoprotein during carbohydrate induction in man. *J. Lab. Clin. Med.* **75:** 206–211.

35. Ruderman, N. B., A. L. Jones, R. M. Krauss, and E. Shafrir. 1971. A biochemical and morphologic study of very low density lipoproteins in carbohydrate-induced hypertriglyceridemia. *J. Clin. Invest.* **50:** 1355–1368.
36. Robertson, R. P., D. J. Gavareski, J. D. Henderson, D. Porte, Jr., and E. L. Bierman. 1973. Accelerated triglyceride secretion. A metabolic consequence of obesity. *J. Clin. Invest.* **52:** 1620–1626.
37. Schonfeld, G., and B. Pfleger. 1971. Utilization of exogenous free fatty acids for the production of very low density lipoprotein triglyceride by livers of carbohydrate-fed rats. *J. Lipid Res.* **12:** 614–621.
38. Bagdade, J. D., D. Porte, Jr., and E. L. Bierman. 1968. Hypertriglyceridemia. A metabolic consequence of chronic renal failure. *N. Engl. J. Med.* **279:** 181–185.
39. Ruderman, N. B., K. C. Richards, V. V. de Bourges, and A. L. Jones. 1968. Regulation of production and release of lipoprotein by the perfused rat liver. *J. Lipid Res.* **9:** 613–619.
40. Heimberg, M., and H. G. Wilcox. 1972. The effect of palmitic and oleic acids on the properties and composition of the very low density lipoprotein secreted by the liver. *J. Biol. Chem.* **247:** 875–880.
41. Levy, R. I., D. W. Bilheimer, and S. Eisenberg. 1971. The structure and metabolism of chylomicrons and very low density lipoproteins (VLDL). *In* Plasma Lipoproteins. R. M. S. Smellie, editor. Academic Press, New York. 3–17.
42. Lux, S. E., R. I. Levy, A. M. Gotto, and D. S. Fredrickson. 1972. Studies on the protein defect in Tangier disease. Isolation and characterization of an abnormal high density lipoprotein. *J. Clin. Invest.* **51:** 2505–2519.
43. Fielding, C. J., and P. E. Fielding. 1971. Purification and substrate specificity of lecithin–cholesterol acyl transferase from human plasma. *FEBS Lett.* **15:** 355–358.
44. Herbert, P. N., R. M. Krauss, H. G. Windmueller, and R. S. Shulman. 1973. Purification of the rat apoprotein activator 441 of lipoprotein lipase. *Circulation.* **48(Suppl. 4):** IV-112. (Abstr.)
45. Havel, Richard J., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32–38.
46. Schonfeld, G., C. Felski, and C. Manning. 1973. The apoprotein composition of plasma chylomicrons during fat absorption in man. *Circulation.* **18(Suppl. 4):** IV-112. (Abstr.)
47. Brown, V. W., and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem. Biophys. Res. Commun.* **46:** 375–382.
48. Havel, R. J., C. J. Fielding, T. Olivecrona, V. G. Shore, P. E. Fielding, and T. Egelrud. 1973. Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoprotein lipase from different sources. *Biochemistry.* **12:** 1828–1833.