Circulating lipids and lipoproteins in glycogen storage disease type I with nocturnal intragastric feeding

Emile Levy,[1,*] Louise A. Thibault, Claude C. Roy, Moise Bendayan, Guy Lepage, and Jacques Letarte

Centre de Recherche, Hôpital Sainte-Justine, Departments of Pediatrics, Medicine, and Anatomy, Université de Montréal, Québec, Canada, and Department of Pediatrics and Gastroenterology,[*] Shaare Zedek Hospital, Jerusalem, Israel

Abstract With the advent of nocturnal intragastric feeding which protects against acute metabolic complications and promotes growth, patients with glycogen storage disease type I are attracting less attention. However, several biochemical alterations persist and suggest that the long-term risk of atherosclerotic heart disease remains high. Persisting hypertriglyceridemia and hypercholesterolemia were found in seven glycogen storage disease type I subjects, six of them following 5–6 yr of nocturnal intragastric feeding. When compared to ten age-matched controls, the patients showed significantly ($P < 0.001$) higher low density lipoprotein cholesterol (LDL-C) (247.7 ± 46.8 vs. 115.3 ± 5.0 mg/dl) and lower high density lipoprotein cholesterol (HDL-C) (26.4 ± 3.4 vs. 55.8 ± 2.9 mg/dl). Triglyceride (TG) enrichment with cholesteryl ester depletion characterized the lipoprotein classes. The diameters of very low density lipoproteins (VLDL) and LDL were larger, while that of HDL was smaller and consistent with the predominance of the HDL_3 subclass and a lower apoA-I/apoA-II ratio. The raised levels of TG appeared attributable not only to the well-described lipogenesis, but also to impaired catabolism of fat, as evidenced by the significantly ($P < 0.001$) decreased activity of both peripheral lipoprotein lipase (3.17 ± 0.43 vs. 14.15 ± 0.50 μmol FFA · ml^{-1} · hr^{-1}) and hepatic lipase (1.88 ± 0.30 vs. 4.83 ± 0.90). This may well explain the high concentration of intermediate density lipoprotein (IDL) and the impaired conversion of HDL_3 to HDL_2. Low apoC-II/apoC-III_1 could be related to defective lipoprotein lipase activity. These data suggest that glycogen storage disease type I patients on nocturnal intragastric feeding remain at risk for atherosclerosis and its complications.— **Levy, E., L. A. Thibault, C. C. Roy, M. Bendayan, G. Lepage, and J. Letarte.** Circulating lipids and lipoproteins in glycogen storage disease type I with nocturnal intragastric feeding. *J. Lipid Res.* 1988. **29:** 215–226.

Supplementary key words VLDL • IDL • LDL • HDL • HDL_2 • HDL_3 • lipoprotein lipase • hepatic lipase • apoB • apoC-II • apoC-III • apoA-I • apoA-II

Glycogen storage disease type I (GSD-I) is an inborn error of carbohydrate metabolism characterized by glycogen and lipid accumulation mainly in the liver, hypoglycemia, lactacidemia, and hyperuricemia (1). In addition, persistently high levels of triglycerides and total cholesterol have been described (2–6). A few studies have reported increased concentrations of very low density and low density lipoproteins but only partial chemical analyses have been carried out (5–7).

Thus, complete characterization of lipoprotein abnormalities, with regard to the core and the surface shell components on the one hand and to particle size and apoprotein pattern by different gel preparation on the other, were not performed.

Continuous nocturnal intragastric feeding (NIF) in combination with frequent daytime feedings reportedly leads to an effective improvement in clinical and biochemical abnormalities associated with GSD-I (8–10). However, close examination of the literature reveals a complete lack of description of lipoprotein composition and metabolism following introduction of this new therapeutic approach. Therefore, it was of interest to study lipoprotein composition in patients with GSD-I managed with this mode of treatment. Because of the relationship between hyperlipoproteinemias and coronary heart disease (11, 12), special attention was focused on the risk factors in view of their implication and the possibility of mitigating them.

In our study, lipids and lipoproteins were quantified in patients with GSD-I. A full characterization of the different classes of lipoproteins was carried out. Because of the

Abbreviations: NIF, nocturnal intragastric feeding; GSD-I, glycogen storage disease type I; HDL, high density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; CM, chylomicrons; TC, total cholesterol; FC, free cholesterol; PL, phospholipid; TG, triglyceride; CE, cholesteryl ester; FFA, free fatty acids; HL, hepatic lipase; LPL, lipoprotein lipase.

[1]Address correspondence to: Emile Levy, Ph.D., Centre de Recherche, Hôpital Sainte-Justine, 3175 Chemin Sainte-Catherine, Montréal, Québec, Canada, H3T 1C5.

close relationship between hyperlipidemia and interaction between lipoproteins, circulating postheparin lipoprotein and hepatic lipase were determined.

METHODS

Subjects studied

Seven patients with a diagnosis of GSD-I, documented by abnormal storage of liver glycogen and by glucose-6-phosphatase deficiency, were studied. Six of them on diazoxide (5 mg/kg per day) were studied before and during NIF. NIF consisted of the intragastric infusion of a high carbohydrate formula (Vivonex HN, Norwich Eaton) at night (one-third of the estimated caloric requirements) with frequent daytime feedings. The composition of the daily diet was 60–70% carbohydrate, 15–20% protein, and the rest was fat. Patient compliance with the dietary treatment was evaluated closely by the team nutritionist during many follow-up visits through regular dietary recall of energy intake and assessment of compliance to the diet. All the patients successfully complied with the nightly placement of the nasogastric tube and respected a schedule of frequent daytime meals. The long-term follow-up (4–6 yr) showed a distinct improvement in their clinical condition, growth, and biochemical abnormalities. Muscle strength improved and there was correction of the chronic metabolic acidosis and of the clotting defect related to platelet function. A significant increase (8.0 ± 8.4 to 22.4 ± 3.4 mg/dl, $P < 0.001$) of glucose levels measured 4 hr after a feeding was observed. It was accompanied by a significant decrease ($P < 0.05$) of lactic acid from 94.8 ± 7 to 58 ± 10 mg/dl, of uric acid from 13.5 ± 1.4 to 8.6 ± 1.0 mg/dl, and of SGPT from 193 ± 49 to 52 ± 11.8 U/l. Both insulin and growth hormone levels showed an elevation from 4.8 ± 0.85 to 18.8 ± 3.2 μU/ml ($P < 0.05$) and from 1.23 ± 0.46 to 4.09 ± 0.97 ng/ml ($P < 0.05$), respectively.

The patients were hospitalized a few days before the special studies to be reported. They were given their calculated caloric requirements. Venous blood samples were collected after 4 hr of fasting following NIF. Ten age-matched healthy children and adolescents were used as controls. Informed consent was obtained from the patients or their parents and permission to conduct the study was obtained from the Ethics Committee of l'Hôpital Sainte-Justine.

Isolation of lipoproteins

Blood was collected in 1 mM disodium EDTA and 0.05% sodium azide from GSD-I patients and controls. Plasma was separated by low speed centrifugation (3,000 rpm, 30 min) at 4°C. The lipoprotein fractions were isolated by sequential ultracentrifugation according to Havel, Eder, and Bragdon (13) with a Ti-50 rotor in a Beckman Model L5-65 ultracentrifuge. After removal of chylomicrons (CM) by preliminary centrifugation (25,000 rpm, 30 min), very low density (VLDL), low density (LDL), and intermediate density (IDL) lipoproteins were separated at densities of 1.006 g/ml, 1.019 g/ml, and 1.063 g/ml, respectively, centrifuging at 100,000 *g* for 18 hr at 5°C. The high density lipoprotein (HDL) fraction was obtained by adjusting the LDL infranatant to d 1.21 g/ml and by centrifuging for 48 hr. Each lipoprotein fraction was washed by its equilibrium density and dialyzed exhaustively against 0.15 M NaCl, 0.001 M EDTA, pH 7.0, at 4°C. In a second phase of this study, HDL subclasses (HDL_2, d 1.125 g/ml and HDL_3, d 1.21 g/ml) were isolated.

Lipid and lipoprotein analysis

Lipoprotein-protein (PR) was determined according to Lowry et al. (14) with bovine serum albumin as a standard. Phospholipids (PL) were measured by the Bartlett method (15) and triglycerides (TG) by a commercial kit (Boehringer Mannheim, Montreal). Total (TC) and free (FC) cholesterol were quantitated by the oxidase-esterase method (16) using the Boehringer kit. Cholesteryl ester (CE) was calculated as the difference between total and unesterified cholesterol. HDL-cholesterol (HDL-C) was measured after precipitation of very low and low density lipoproteins with phosphotungstic acid (17). LDL-cholesterol (LDL-C) was determined using polyvinylsulfate (Boehringer Mannheim) (18). VLDL cholesterol was calculated from the difference between cholesterol in the polyvinylsulfate supernatant and HDL-cholesterol. The apolipoprotein (apo) content of lipoproteins was examined by electrophoresis in SDS-polyacrylamide gel (SDS-PAGE) (19) or subjected to tetramethylurea gel electrophoresis (TMU gel) (20). The densitometric distribution of apolipoproteins was assayed as described previously (21). Plasma was also subjected to agarose gel electrophoresis as described previously (22). Electron microscopy of lipoprotein particles was performed on a Zeiss EM-10, using negative staining with 1% phosphotungstic acid (pH 7.2) as described previously (23). The diameter of 500–800 particles was then determined. Total fatty acids (FA) and free fatty acids (FFA) were measured with an improved method described previously (24). The technique consists of a 1-hr direct transesterification procedure, carried out in methanol–benzene 4:1 with acetyl chloride. L-Carnitine (25) and apoB (26) were also determined.

Lipolytic activity measurement

Postheparin plasma was taken by venous puncture 10 min after an intravenous injection of heparin (10 units/kg body weight) (27). Lipolytic activity was measured with an emulsion of tri[1-^{14}C]oleoyl glycerol as substrate (28). Hepatic triglyceride lipase (HL) activity was assayed in the presence of protamine sulfate which was verified to

inhibit completely peripheral lipoprotein lipase (LPL). Extraction of FFA was performed by the procedure of Belfrage and Vaughan (29) as previously described (30).

Statistical analysis

All values were expressed as the mean ± standard error (SEM). Statistical differences were assessed by the Student's two-tail *t*-test.

RESULTS

Plasma lipids

The different characteristics of GSD-I subjects and the changes in their TG and TC levels under NIF are shown in **Table 1.** Hypertriglyceridemia and hypercholesterolemia, although reduced by the treatment, were not restored to normal. Thus, at the time of this study, abnormally elevated values of TG, TC, and apoB were observed (**Table 2**). In contrast to FFA levels, which did not differ from controls, total fatty acids were markedly increased (941.6 ± 192.5 vs. 238.1 ± 11.0 mg/dl). Carnitine levels were also found to be higher. The percentage esterification of cholesterol was significantly reduced in patients when compared to controls (60.9 ± 3.1 vs. 74.1 ± 0.7%). As expected, because of the high plasma cholesterol concentration, VLDL- and LDL-cholesterol were increased (**Table 3**). This 50% increase of VLDL-C and LDL-C was associated with a 53% reduction of HDL-C. Differences between GSD-I patients and controls became more marked when these parameters were expressed as ratios. The HDL-C to TC ratio was 0.10 ± 0.02 versus 0.30 ± 0.01 in controls. The corresponding ratio for HDL-C to LDL-C in patients was 0.13 ± 0.02 and 0.49 ± 0.02 in controls. The latter as well as HDL-C levels were mostly below the 5th percentile for age- and sex-matched controls of the large LRC study.

Using semiquantitative agarose gel electrophoresis, an α-lipoprotein proportion averaging 2.8 ± 0.99% was found before NIF. This α-lipoprotein proportion was markedly ($P < 0.01$) elevated to 9.54 ± 2.27% during NIF therapy (**Fig. 1**). However, it remained significantly lower ($P < 0.001$) than the mean of controls 35.0 ± 5.1% (Fig. 1). In association with the diminished percentage of α-lipoprotein, an excessive proportion of pre-β- and β-lipoprotein was recorded. The heterogeneity of the electrophoretic patterns on agarose gel is illustrated in Fig. 1. In some cases of GSD-I, there was a strong pre-β-lipoprotein peak and a reduced β peak, while other individuals presented only one major peak in the β region. These lipoprotein profiles during NIF were not substantially different from those observed prior to NIF, except for α-lipoproteins reported before.

Lipid and apoprotein composition of lipoproteins

By sequential ultracentrifugation, plasma lipoproteins of GSD-I were isolated at densities 1.006 g/ml, 1.063 g/ml, and 1.21 g/ml which correspond to VLDL, LDL, and HDL, respectively (**Table 4**). All these classes were triglyceride-enriched and poorer in CE. Free cholesterol (FC) was also lower except in HDL. PL were normal in VLDL and LDL, and significantly higher in HDL. Protein was increased in LDL only. As expected from these changes in lipoprotein components, particle size was also affected. The diameter of VLDL was greater (398 ± 18 vs. 309 ± 12 Å, $P < 0.05$), although differences with respect to the ratios TG/PL and TG + CE/FC + PL + PR were not significant between GSD-I and controls. It should be noted that the mass ratio of core constituents (TG + CE) to surface constituents (FC + PR + PL) can be used to make inference on the size of spherical lipoprotein particles. In general, lighter and larger populations are relatively enriched with core components as compared to denser and smaller particles. The calculated value of these ratios and diameter measurements indicated

TABLE 1. Characteristics of GSD-I subjects

Subject	Sex	Age	Height	Height-Age	Weight-Age	Hepatomegaly Z Score[a]	Duration of NIF[b]	TG Range		TC Range	
								− NIF	+ NIF	− NIF	+ NIF
		yr, month	*cm*	*yr, month*	*yr, month*		*yr*	*mg/dl*		*mg/dl*	
1	F	10, 3	117.9	7	6, 3	25.2	6	1290 ± 117	776 ± 75[c]	353 ± 32	313 ± 12[c]
2	F	18, 4	156.9	14	17	18.1	5	577 ± 37	207 ± 13[c]	318 ± 30	200 ± 9[c]
3	M	22, 6	159.4	15, 4	14	26.6	5	2444 ± 142	1088 ± 147[c]	399 ± 25	320 ± 23[c]
4	M	20	158.6	15, 6	16	22.2	5	1147 ± 55	703 ± 29[c]	287 ± 8	224 ± 14[c]
5	F	17, 7	136.4	10, 4	12, 5	19.7		565 ± 52		220 ± 8	
6	F	15, 9	144.4	11, 8	12, 10	15	4	1470 ± 85	491 ± 46[c]	415 ± 24	280 ± 7[c]
7	F	26	145.4	11, 9	13, 8	19.6	5	4016 ± 146	1646 ± 139[c]	1061 ± 49	497 ± 29[c]

[a] Z score is the index demonstrating the deviation of the actual liver weight from the ideal weight, calculated according to McAfee et al. (31).
[b] NIF, nocturnal intragastric feeding.
[c] Values of TG and TC are mean ± SEM. Differences between untreated and treated patients are significant at a level of at least $P < 0.05$.

TABLE 2. Plasma lipid, apoB, and carnitine determinations

Subject	Total FA	FFA	TG	PL	TC	FC	CE as % of TC	ApoB[a]	Carnitine
			mg/dl				%	*mg/dl*	*μmol/dl*
GSD-I									
1	1154	64.1	1025	550	344	129	63	238	7.7
2	379	39.1	161	410	222	61	73	159	5.8
3	1730	70.7	1465	620	400	189	53	345	6.0
4	1003	32.6	930	490	250	113	54	111	9.0
5	678	52.4	533	370	194	73	62	185	7.7
6	705	48.0	491	390	280	89	69	277	5.9
7			3240	1030	650	313	52	294	6.9
Mean ± SEM	941.6 ± 192.5	51.1 ± 6.0	1121 ± 338	551 ± 87	334 ± 59	138 ± 33	60.9 ± 3.1	230 ± 31	7.0 ± 0.4
Controls									
Mean ± SEM	238.1 ± 11.0	34.2 ± 5.1	65 ± 3	194 ± 12	179 ± 9	47 ± 3	74.1 ± 0.7	112 ± 19	4.6 ± 0.5
$P <$	0.005	NS	0.02	0.005	0.02	0.02	0.001	0.005	0.005

Data are means ± SEM of the seven GSD-I patients following NIF and ten controls who participated in the study.
[a]ApoB was determined by radioimmunoassay.

that LDL particles were larger (242 ± 3 vs. 219 ± 3 Å, $P < 0.005$) and HDL smaller (85 ± 4 vs. 106 ± 12 Å, $P < 0.05$). In view of the fact that LDL isolation at d 1.006 to 1.063 g/ml included IDL, the LDL alterations could account for IDL inclusion. So, in a second step in this study, IDL and LDL were isolated at d 1.006 to 1.019 g/ml and 1.019 to 1.063 g/ml, respectively, and lipid and protein analyses were carried out. Persistent compositional abnormalities were observed in pure LDL fraction (Table 4). However, on the basis of core constituents to surface constituents, the LDL fraction free of IDL seemed to contain smaller particles than the corresponding plasma LDL of controls (0.59 ± 0.03 vs. 0.91 ± 0.06, $P < 0.005$). When the data from GSD-I IDL fractions were compared to those of IDL from normal subjects, differences relative to the composition and to the size were found. The contribution of TG to total IDL mass increased while that of CE and FC decreased. No consistent changes were found in PL and PR. Hence, the weight ratio of TG/PL and TG + CE/FC + PL + PR increased, inferring, thereby, that the IDL particles of GSD-I patients are larger.

The 10% SDS-PAGE showed an apolipoprotein distribution in isolated VLDL, which differed from that obtained from normal subjects (**Fig. 2A**). More specifically, there were visible bands close to apoB-100 as well as to apoE. In addition, both apoB-100 and apoE appeared to be more abundant. To assess the contribution of intestinal lipoproteins to total VLDL, SDS-PAGE using 4% acrylamide was performed. The presence of low molecular weight apoB (B-48) was demonstrated (Fig. 2B). In LDL isolated from patients and controls there were no consis-

TABLE 3. Lipoprotein cholesterol levels with HDL/LDL and HDL/TC ratios

Subject	Cholesterol					Percentiles[a]		
	VLDL	LDL	HDL	HDL/LDL	HDL/TC	HDL	HDL/LDL	HDL/TC
			mg/dl					
GSD-I								
1	60	253	31	0.12	0.09	<5	<5	<5
2	22	161	39	0.24	0.18	>10	<15	= 10
3	72	295	33	0.11	0.08	>10	<5	<5
4	39	184	24	0.13	0.10	<5	<5	<5
5	43	135	16	0.12	0.08	<5	<5	<5
6	49	206	28	0.14	0.10	<5	<5	<5
7	136	500	14	0.03	0.02	<5	<5	<5
Mean ± SEM	60.1 ± 14.0	247.7 ± 46.8	26.4 ± 3.4	0.13 ± 0.02	0.10 ± 0.02			
Controls (n = 15)								
Mean ± SEM	14.2 ± 1.4	115.3 ± 5.0	55.8 ± 2.9	0.49 ± 0.02	0.30 ± 0.01	50	50	50
$P <$	0.001	0.001	0.001	0.001	0.05			

Biochemical parameters were measured in GSD-I patients following NIF.
[a]Deduced from prevalence study reported by Lipid Research Clinics (LRC) (32, 33).

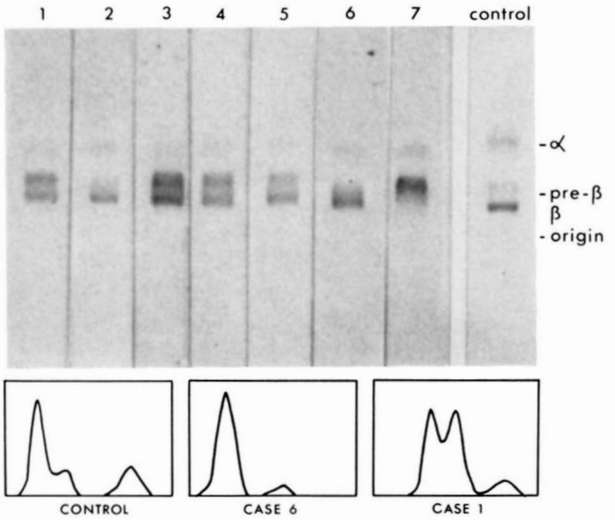

Fig. 1. Lipoprotein electrophoresis of plasma from a normal subject (c) and from GSD-I patients (1-7) (upper panel). With this technique, β-lipoproteins, pre-β-lipoproteins (pre-β), and α-lipoproteins (α) are visualized (by oil red O). The integrated scan (lower panel) provides evidence for *1*) lower proportion (%) of α-fraction in GSD-I when compared to controls; and *2*) different lipoprotein profiles in GSD-I.

tent differences. The separation of VLDL-apoC on a TMU gel is illustrated in **Fig. 3**. In six separate experiments, the VLDL from GSD-I patients clearly showed a markedly elevated proportion of apoC-III, which led to a decrease of the C-II/C-III$_1$ ratio calculated from densitometric scans (Fig. 3). Significant differences were equally observed in the A-I/A-II ratio from integrated densitometric tracings of HDL submitted to 15% SDS-PAGE (**Fig. 4**). A representative and reproducible densitometric scan of the apoC and apoA regions is shown in **Fig. 5** which illustrates the good resolution of apolipoproteins obtained by polyacrylamide gel electrophoresis. In view of the alterations in the composition of LDL and HDL, we examined the level of IDL and the subpopulations of HDL in a second part of the study. **Table 5** shows that GSD-I patients had increased IDL concentrations (92.5 ± 15.4 vs. 34.0 ± 5.3 mg/dl). The lower values of total HDL (15.5 ± 25.5 vs. 229.0 ± 5.8 mg/dl) were totally accounted for by the pronounced decrease of HDL_2 (50.0 ± 22.0 vs. 130.0 ± 7.6 mg/dl).

Lipoprotein lipase activity

Hypertriglyceridemia was associated with a 78 ± 2.9% decrease of total postheparin lipolytic activity (3.11 ± 0.4 vs. 14.15 ± 0.50 μmol $FFA \cdot ml^{-1} \cdot hr^{-1}$ in controls) in response to NIF (**Table 6**). The most affected was hepatic lipase (HL) activity. Expressed as a percent of decrease from the mean of control values, HL activity was 86.2 ± 2.4% versus 61.2 ± 6.2% for LPL activity. Moreover, a change in the pattern was observed in GSD-I. While HL was the predominant activity in the plasma of controls, LPL activity was the major component in the plasma of GSD-I. This was well reflected by the HL/LPL ratio

TABLE 4. Chemical composition of lipoprotein

Lipoprotein	Composition					Weight Ratios	
	TG	CE	FC	PL	PR[a]	$\frac{TG}{PL}$	$\frac{TG+CE}{FC+PL+PR}$
	mg/100 mg of lipoprotein						
VLDL (d 1.006 g/ml)							
Controls	56.1 ± 1.0	10.5 ± 1.1	5.7 ± 0.3	16.5 ± 0.2	10.1 ± 0.7	3.40 ± 0.07	2.15 ± 0.24
GSD-I	62.4 ± 1.9	6.5 ± 0.1	4.8 ± 0.2	16.9 ± 0.2	9.5 ± 0.5	3.70 ± 0.16	2.09 ± 0.21
P <	0.02	0.005	0.05	NS[b]	NS	NS	NS
IDL (1.006<d<1.019 g/ml)							
Controls	21.6 ± 1.4	21.8 ± 2.1	7.4 ± 0.7	31.6 ± 3.2	17.5 ± 0.3	0.74 ± 0.10	0.79 ± 0.08
GSD-I	42.4 ± 1.9	7.4 ± 0.4	5.8 ± 0.4	26.2 ± 1.0	18.2 ± 0.3	1.62 ± 0.08	1.01 ± 0.07
P <	0.01	0.001	0.05	NS	NS	0.001	NS
LDL (1.019<d<1.063 g/ml)							
Controls	6.4 ± 0.7	40.3 ± 1.5	9.5 ± 0.7	22.5 ± 0.6	21.3 ± 1.2	0.28 ± 0.02	0.91 ± 0.06
GSD-I	22.0 ± 3.3	15.2 ± 1.9	6.0 ± 0.4	27.2 ± 1.0	29.6 ± 2.6	0.80 ± 0.11	0.59 ± 0.03
P <	0.01	0.001	0.005	0.005	0.02	0.001	0.005
LDL (1.006<d<1.063 g/ml)							
Controls	7.8 ± 0.3	32.4 ± 1.3	8.4 ± 0.4	24.1 ± 0.7	26.8 ± 0.7	0.32 ± 0.01	0.68 ± 0.03
GSD-I	27.1 ± 1.8	20.8 ± 1.2	6.2 ± 0.2	23.6 ± 0.8	22.2 ± 0.3	1.17 ± 0.12	0.92 ± 0.03
P <	0.001	0.001	0.001	NS	0.001	0.001	0.001
HDL (1.063<d<1.21 g/ml)							
Controls	4.9 ± 0.7	20.5 ± 0.2	3.2 ± 0.2	24.2 ± 1.1	47.2 ± 1.4	0.20 ± 0.03	0.36 ± 0.01
GSD-I	8.2 ± 0.7	11.5 ± 1.1	2.6 ± 0.3	30.4 ± 0.6	47.3 ± 1.4	0.27 ± 0.03	0.25 ± 0.03
P <	0.005	0.001	NS	0.001	NS	NS	0.001

Lipoprotein characterization was carried out in GDS-I patients following NIF.
[a]Protein.
[b]NS, not significant.

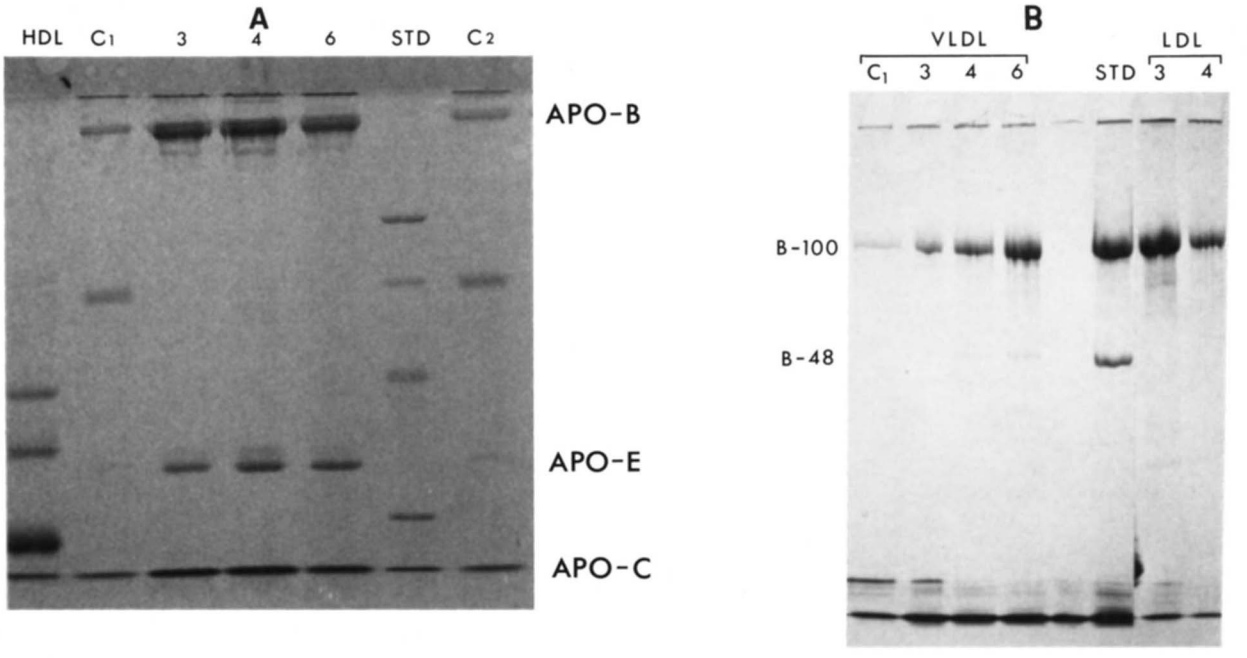

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis showing the apolipoprotein composition of VLDL and LDL from patients 3, 4, and 6 and controls (C_1 and C_2). (A) represents the pattern obtained with 10% gel. About 30 μg of VLDL delipidated with ethanol–ether 3:1 was applied on the gel under reducing conditions (1% β-mercaptoethanol). Rat HDL and molecular weight standards (STD) were used as reference proteins. STD were phosphorylase B (92,500), BSA (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), and lysozyme (14,400). (B) represents the pattern of VLDL and LDL obtained with 4% gel. The STD containing B-100 and B-48 was isolated from patients with type I hyperlipidemia.

(0.69 ± 0.09 vs. 2.48 ± 0.76 in controls). NIF seemed to induce a significant increase of both hepatic and extrahepatic lipoprotein lipase activities, but the proportional contribution of each to the total postheparin lipolytic activity was not changed significantly. We also measured the in vitro postheparin plasma LPL and HL activities in some GSD-I patients after addition of inactivated plasma from normal subjects. Both enzyme activities increased

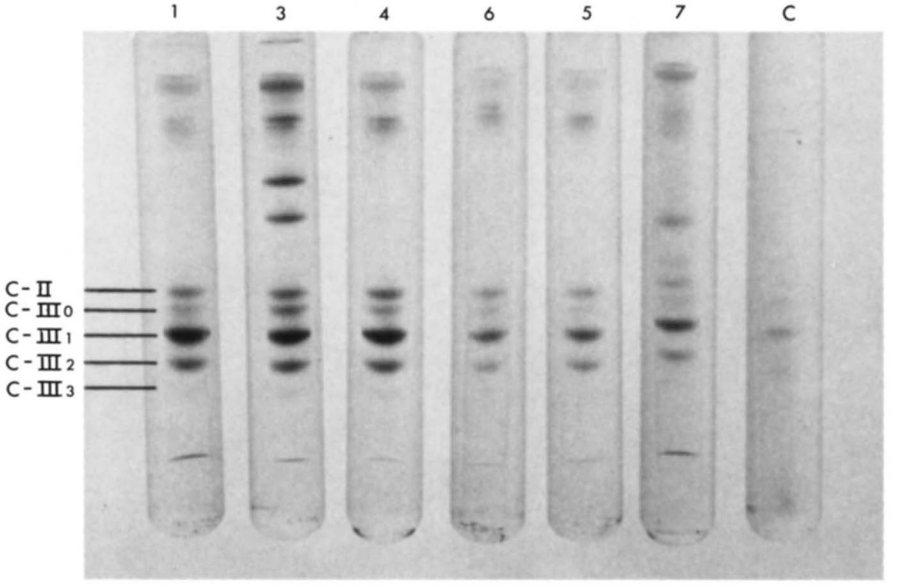

Fig. 3. Separation of apoVLDL on 10% polyacrylamide gel containing 8 M urea. After delipidation, solubilized proteins were electrophoresed and the staining was performed with Coomassie Blue. A typical densitometric tracing of apoproteins is presented in Fig. 5. The C-II/C-III_1 ratios calculated from the corresponding scans are significantly different ($P < 0.05$): GSD-I = 0.28 ± 0.06 (n = 6); controls = 0.34 ± 0.08 (n = 12).

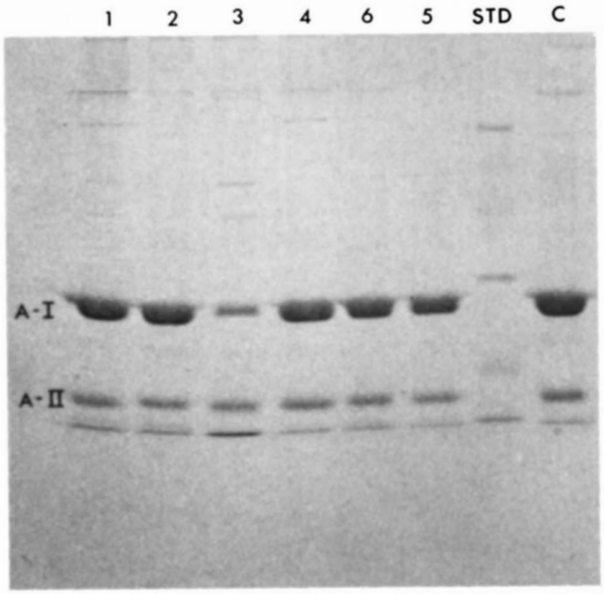

Fig. 4. Apolipoprotein patterns of HDL fraction (d < 1.21 g/ml) on 15% polyacrylamide gel containing 0.1% SDS. The location of apolipoprotein species of HDL from patients 1–6 and one control (C) were identified by comparison with molecular weight standards detailed in Fig. 2. A typical densitometric tracing is shown in Fig. 5. The A-I/A-II ratios calculated from the corresponding scans are significantly different ($P < 0.001$): GSD-I = 3.09 ± 0.48; controls = 5.41 ± 0.37.

considerably by 100 to 200%, suggesting that the enzyme protein concentration was normal.

DISCUSSION

The present study describes abnormalities in plasma lipids, in the composition and size of lipoproteins, as well as in some factors that contribute to their interconversion in seven GSD-I patients, six of whom were on long term continuous NIF.

There is good evidence that frequent daytime feeding combined with nocturnal intragastric feeding leads to a distinct improvement of growth, metabolic complications, and biochemical features. Although beneficial, the treatment has a limited capacity to return plasma lipids and lipoproteins towards normal. The biochemical probes and electron microscopy studies identified several abnormalities. Total FA, FFA, TG, PL, TC, and FC were markedly elevated despite the compliance of the patients with the nightly placement of the nasogastric tube and a schedule of frequent daytime meals. Our present investigation confirms previous reports that hyperlipidemia is only partly corrected with the treatment of glucose-6-phosphatase-deficient patients by NIF (8, 34–37). These studies have already reported that following treatment, TG concentrations were substantially lowered, but remained elevated to varying degrees.

Of major importance was the significant increase in VLDL-C and LDL-C coupled with a decrease in HDL-C in the GSD-I patients. This was documented in two ways: a direct measurement and agarose gel electrophoresis. Most epidemiological studies have shown that two general mechanisms are associated with risk of atherosclerosis: one involves elevated plasma levels of the atherogenic lipoprotein (LDL) and the second involves a low HDL concentration resulting in a defective removal of intracellular cholesterol. The GSD-I patients met these criteria and, in addition, the magnitude of their supposedly antiatherogenic subfraction HDL_2 was affected. It was also possible to note significant differences in the chemical lipoprotein composition. VLDL and LDL (d 1.006–1.063 g/ml) were TG-enriched. An increased proportion of TG was largely responsible for their greater size (**Fig. 6**) and lighter density given a relative decrease in CE. Since this LDL population also included IDL that were found to be increased threefold over control values, pure LDL (d 1.019–1.063 g/ml) were isolated in a second phase of this study. The same abnormalities were observed but, as expected, the size of pure LDL particles was smaller. On a weight percent basis, the HDL class also differed in size and lipid content. They clearly had more TG and less CE than controls. The observed electron microscopic size of HDL agreed well with TG + CE/FC + PL + PR ratio and revealed a smaller diameter in patients. These observations could be due to the predominance of the smaller and denser HDL_3 subpopulation. A virtually identical situation has been described in cases of severe hypertriglyceridemia. The HDL particles are smaller in size and they are cholesteryl ester-poor as well as triglyceride-rich (38, 39).

Why is the percentage of cholesteryl ester molecules reduced in plasma as well as in all lipoprotein fractions, and why is HDL_2 selectively deficient in GSD-I patients? The results of this study could suggest a direct relationship between the activity of LPL and HDL-C as described before in other systems and subjects by Nikkila et al. (40–42) and Huttunen et al. (43). However, the data fit more conveniently with the concept that nearly all the variations of plasma HDL concentrations are due to fluctuations in HDL_2 (44, 45). Hence the combination of lower HDL_2 and LPL levels leads to the speculation that the mechanism of HDL_3 to HDL_2 conversion is slowed because of a limited incorporation of molecules released from the surface of lipolyzed TG-rich lipoproteins into HDL_3 (46). Such a reduced transfer of surface components from VLDL to HDL_3 during lipolysis concomitantly makes less FC available for esterification and redistribution. It is known that efficient esterification of cholesterol is dependent on the activity of LCAT which is stimulated by apoA-I (47). The concentration of the latter was reported to be normal in GSD-I subjects (48).

The plasma clearance of TG-rich VLDL appears to be impaired in GSD-I patients. Both LPL and HL were

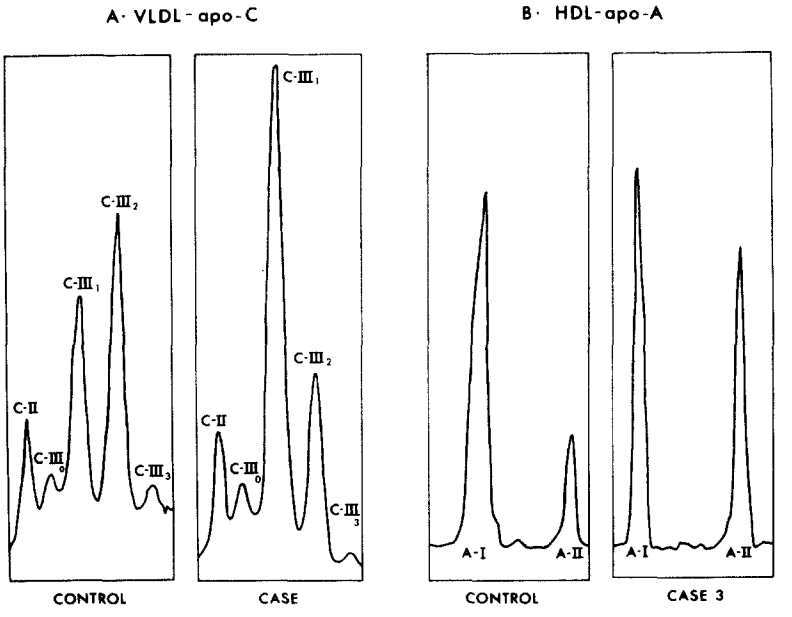

Fig. 5. A typical densitometric tracing obtained from VLDL-apoC in 10% polyacrylamide gel containing 8 M urea (A) and from HDL-apoA in 15% SDS-PAGE (B).

decreased and evidently did not play their usual central role in lipoprotein catabolism. In view of the possible competition of the high endogenous TG with the [^{14}C]triolein used for the quantification of postheparin plasma lipolytic activity, we proceeded with different and prudent attempts to establish and clarify this important point definitively. Although the TG were discarded by ultracentrifugation (d 1.006 g/ml), the patients' plasma disclosed severely affected LPL activity. Moreover, we never obtained a close correlation between LPL activity and TG levels in GSD-I patients. Finally, when we submitted some pre- and postheparin plasma to gas–liquid chromatography, after 1 hr incubation at 37°C, FFA determination revealed a low activity of LPL. Of interest in this investigation was the finding that HL was more affected than LPL as evidenced by the HL LPL ratio. Without changing the latter ratio, NIF appeared to improve lipolytic activity but it did not restore it to normal.

Since the role of insulin in activating LPL by increasing the synthesis of enzyme protein has been demonstrated (49, 50), perhaps an improvement in the overall control of insulin secretion resulting from NIF could be at play. It should be pointed out that our GSD-I patients were treated before and during NIF therapy with diazoxide, a drug that is a powerful inhibitor of insulin secretion (51). Diazoxide is an anti-hypertensive benzothiadazine drug which is capable of elevating blood glucose concentration in intact, pancreactomized, and alloxanized animals (52). Its use in humans was shown to be beneficial in several clinical conditions, namely hypoglycemia in infants (53), hyperinsulinism states (54), glycogen storage disease (55, 56), and even in hyperlipidemia (57). Several observations support pancreatic and extrapancreatic mechanisms in the diabetogenic activity of diazoxide (58). Our patients were treated with a small dose of diazoxide before NIF initiation. This treatment has proved to be useful for the

TABLE 5. IDL and HDL concentrations in GSD-I

Subjects	IDL	HDL_2	HDL_3	Total HDL	HDL_2/HDL_3
		mg/dl			
Controls	34.0 ± 5.3	130.0 ± 7.6	99.0 ± 7.1	229.0 ± 5.8	1.4 ± 0.1
GSD-I	92.5 ± 15.4[a]	50.0 ± 22.0[a]	104.0 ± 30.5	155.0 ± 25.5[b]	0.7 ± 0.3

IDL (d 1.019 g/ml) and HDL subclasses (HDL_2, d 1.125 mg/dl; HDL_3, d 1.21 g/ml) were isolated by preparative ultracentrifugation. The values in the table represent the lipoprotein mass in plasma as calculated by lipid and protein determinations.

[a] $P < 0.01$.

[b] $P < 0.005$.

TABLE 6. Postheparin lipolytic activity

Subject	Total PHLA[a]		LPL[b]		HL[c]		HL/LPL	
	− NIF	+ NIF	− NIF	+ NIF	− NIF	+ NIF	− NIF	+ NIF
	μmoles FFA · ml^{-1} · hr^{-1} (% decrease from mean of control values)							
GSD-I								
1	1.60 (88.7)	3.23 (77.2)	1.02 (78.9)	1.83 (62.1)	0.58 (93.8)	1.40 (85.0)	0.57	0.76
2	0.55 (96.1)	1.05 (92.6)	0.28 (94.2)	0.71 (85.3)	0.27 (97.1)	0.34 (96.3)	0.96	0.48
3	1.16 (91.8)	4.18 (70.4)	0.91 (81.1)	2.25 (54.3)	0.25 (97.3)	1.93 (79.3)	0.27	0.86
4	1.82 (87.8)	4.11 (70.9)	0.93 (80.7)	2.46 (49.1)	0.79 (91.5)	1.65 (82.3)	0.85	0.67
5	2.75 (80.6)		2.08 (56.9)		0.67 (92.8)		0.32	
6	1.03 (92.7)	2.74 (80.6)	0.66 (86.3)	1.40 (71.0)	0.37 (96.0)	1.34 (85.6)	0.56	0.96
7	2.08 (85.3)	3.73 (73.6)	1.41 (70.8)	2.66 (44.9)	0.67 (92.8)	1.07 (88.5)	0.47	0.40
Mean ± SEM	1.55 ± 0.27 (89.0 ± 1.9)	3.17 ± 0.47 (77.5 ± 3.4)	1.04 ± 0.22 (78.4 ± 4.5)	1.88 ± 0.30 (61.2 ± 6.2)	0.51 ± 0.08 (94.5 ± 2.3)	1.29 ± 0.22 (86.2 ± 2.4)	0.57 ± 0.10	0.69 ± 0.09
GSD-I vs. controls $P <$	0.001	0.001	0.001	0.001	0.001	0.001	0.05	0.05
− NIF vs. + NIF $P <$	0.02		0.05		0.01		NS	
Controls Mean ± SEM	14.15 ± 0.50		4.83 ± 0.90		9.32 ± 1.30		2.48 ± 0.76	

[a]PHLA: Postheparin lipolytic activity.
[b]LPL: Extrahepatic lipoprotein lipase.
[c]HL: Hepatic lipase.

management of hypoglycemia and other biochemical manifestations. This same therapy, when combined with frequent daytime feeding and NIF, resulted in increased and more stable plasma glucose levels as well as in the improvement of other biochemical derangements (results not documented here). We never observed side effects except occasional hirsutism. As reported by other workers in experimental and clinical studies, we noted that diazoxide administration led to a substantial fall in plasma TG and cholesterol concentrations (results not shown). Chait et al. (59) have already shown that diazoxide caused a reduction in VLDL production and Eaton and Nye (57) demonstrated that withdrawal of diazoxide resulted in an elevation in primary hypertriglyceridemia. These authors also suggested that the catabolism of VLDL tended to decrease during diazoxide therapy. It is noteworthy that LPL in our patient, while insulin-dependent, was not disturbed by the diazoxide administration. Two patients showed a small LPL increase on diazoxide when compared to the previous period without diazoxide (results not reported). Our experience is that the small dose, while beneficial to the patients, it not sufficient to modify insulin secretion significantly. In fact, the concentration of circulating insulin was normal in our patients. This could explain the lack of association between diazoxide treatment and LPL activity. Finally, the single variable in this study was the addition of NIF. All patients were on diazoxide at the same dose, before and after the dietary change. Thus, whereas diazoxide might potentially influence lipoprotein metabolism, this is an unlikely explanation for our findings.

In order to gain further insight into the relationship between the LPL activity and some of the factors leading to its decrease, two steps were undertaken. One step involved the measurement of apoC-II/apoCIII$_1$ ratio and the second involves lipolytic activity determination after the addition of inactivated plasma. The currently altered apoC-II/apoC-III$_1$ ratio in VLDL could result in decreased LPL activity, as supported by a previous investigation of other pathological states (60) and, hence, defective VLDL catabolism in our patients. From other reports, we know that normalization of the apoC-II/apoC-III ratio parallels a decrease in VLDL-TG as well as an increase in LPL activity (61–64). However, this hypothesis can only explain the decreased LPL activity and does not hold in the case of HL because its activity is totally independent of apoC-II and apoC-III. We have also taken into account the suggestion that FFA could produce LPL inhibition (63). However, in view of normal FFA concentrations found in the present study, this type of inhibition is un-

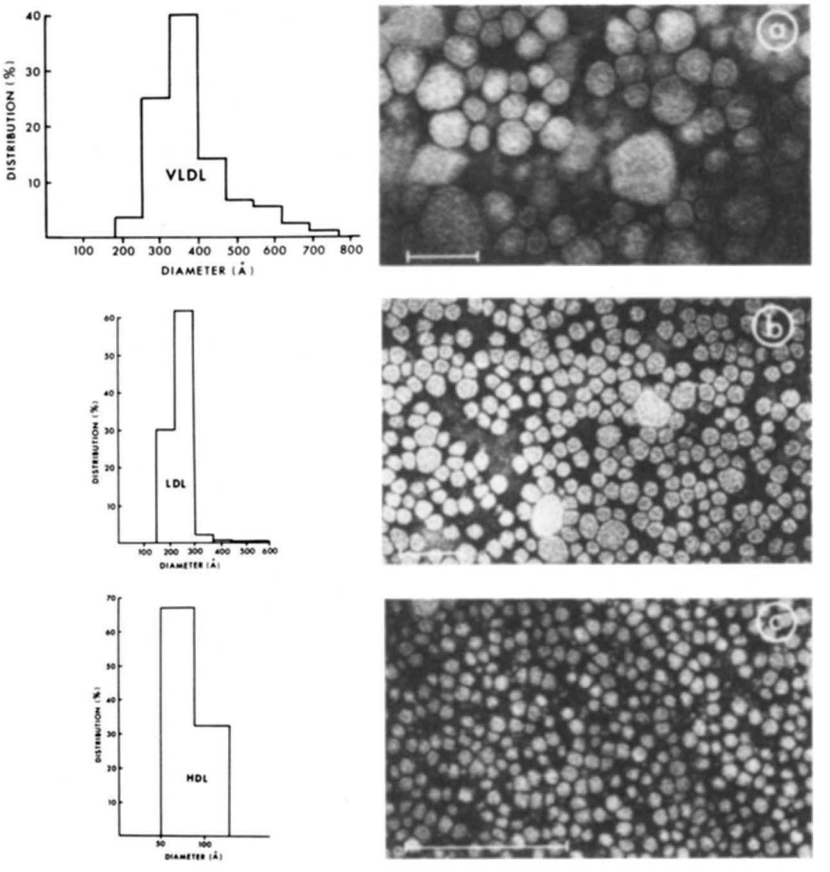

Fig. 6. Representative electron photomicrographs of negatively stained plasma lipoproteins (at right) and frequency distribution of particle diameter in GSD-I patient, case 4 (at left). The lipoprotein fractions are: a) VLDL; b) LDL; and c) HDL. Each bar represents 500 Å. When a minimum of 500 particles from four patients was measured, the mean ± SEM was: VLDL, 394 ± 18 versus 309 ± 12 Å, $P < 0.05$; LDL, 243 ± 3 versus 219 ± 3 Å, $P < 0.05$; and HDL, 85 ± 4 versus 106 ± 2 Å, $P < 0.05$.

likely to be involved. Carnitine levels were appropriately high in order to maintain the high rate of oxidation of long chain fatty acids necessary to prevent increased concentrations of FFA.

As alluded to earlier, hyperlipidemia, as seen in GSD-I patients, may be an important risk factor for heart disease. At this time, we have not found any clinical signs or symptoms of coronary artery disease in our young patients. However, many epidemiologic studies have defined factors associated with increased risk of atherosclerotic diseases long before the sequelae manifest themselves, because atherosclerosis is a disease which develops silently (65). Atherosclerotic changes have been observed in arteries of young asymptomatic children growing up in industrialized countries (66). Therefore, particular attention should be given to all children with hyperlipidemia. In this context, our patients had well-defined biochemical risk factors. First, they had low HDL-C and high LDL-C concentrations. Second, the decrease in HDL-C was accompanied by diminished total HDL and A-I/A-II. Third, the antiatherogenic subfraction HDL_2 was most significantly affected. Finally, they had a significant degree of hypertriglyceridemia which constitutes, in the opinion of some authors, a significant risk factor for coronary artery disease (65). We therefore conclude that, even on NIF, GSD-I patients can be considered at high risk for the development of coronary artery disease. Studies should be carried out with the aim of devising new strategies (dietary and lipid-lowering drugs) in the hope of improving the current management.

We thank Dr. Yves Marcel for performing the measurement of apoB and we appreciate Sylvie Tassé's assistance in manuscript preparation. This project was supported by H. J. Heinz Co. of Canada Ltd. and by the Medical Research Council of Canada.

Manuscript received 8 September 1986, in revised form 26 March 1987, and in re-revised form 23 July 1987.

REFERENCES

1. Howell, R. R. 1978. The glycogen storage diseases. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Frederickson, editors. McGraw Hill, New York. 143–146.
2. Fernandes, J., and N. A. Pikaar. 1969. Hyperlipemia in children with liver glycogen disease. *Am. J. Clin. Nutr.* **22:** 617–627.
3. Forget, P. P., J. Fernandes, and P. Haverkamp-Begemann. 1974. Triglyceride clearing in glycogen storage disease. *Pediatr. Res.* **8:** 114–119.
4. Howell, R. R., D. M. Ashton, and J. B. Wyngaarden. 1962. Glucose-6-phosphatase deficiency in glycogen storage disease: studies on the interrelationship of carbohydrate, lipid and purine abnormalities. *Pediatrics.* **29:** 553–565.
5. Jakovcic, S., A. K. Khachadurian, and D. J. J. Hsia. 1966. The hyperlipidemia in glycogen storage disease. *J. Lab. Clin. Med.* **68:** 769–779.
6. Kolb, F., O. DeLalla, and J. W. Gofman. 1955. The hyperlipidemias in disorders of carbohydrate metabolism: serial lipoprotein studies in diabetic acidosis with xanthomatosis and in glycogen storage disease. *Metabolism.* **4:** 310–317.
7. Rosenfeld, E. L., I. V. Chibisov, I. M. Karmansky, V. A. Tabolin, L. V. Chistova, and A. F. Leontiev. 1980. Serum lipoproteins of patients with glycogen storage disease. *Clin. Chim. Acta.* **102:** 99–104.
8. Greene, H. L., A. F. Slonim, J. A. O'Neill, and I. M. Burr. 1976. Continuous nocturnal feeding for management of type I glycogen storage disease. *N. Engl. J. Med.* **294:** 423–425.
9. Slonim, A. E., A. B. Terry, R. Moran, P. Benke, H. L. Greene, and I. M. Burr. 1978. Differing food composition for nocturnal intragastric therapy in types I and III glycogen storage disease. *Pediatr. Res.* **12:** 512 (abstract).
10. Greene, H., A. E. Slonim, I. M. Burr, and J. R. Moran. 1980. Type I glycogen storage disease: five years of management with nocturnal intragastric feeding. *J. Pediatr.* **96:** 590.
11. Inkeles, S., and D. Eisenberg. 1981. Hyperlipidemia and coronary atherosclerosis: a review. *Medicine* (Baltimore) **60:** 110–123.
12. Eisenberg, S. 1984. High density lipprotein metabolism. *J. Lipid Res.* **25:** 1017–1058.
13. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoprotein in human serum. *J. Clin. Invest.* **34:** 1345–1353.
14. 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.
15. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
16. Patsch, W., S. Sailer, and H. Braunsteiner. 1976. An enzymatic method for the determination of the initial rate of cholesterol esterification in human plasma. *J. Lipid Res.* **17:** 182–185.
17. Lopez-Virella, M. F., P. Stone, S. Ellis, and J. A. Colwell. 1977. Cholesterol determination in high density lipoprotein separated by three different methods. *Clin. Chem.* **23:** 882–891.
18. Schriewer, B., W. Nolte, and G. Assmann. 1985. VLDL apolipoprotein B determination in blood serum following precipitation of LDL with polyvinylsulphate. *J. Clin. Chem. Clin. Biochem.* **23:** 349–353.
19. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
20. Kane, J. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
21. Levy, E., E. Shafrir, E. Ziv, and H. Bar-On. 1985. Composition, removal and metabolic fate of chylomicrons derived from diabetic rats. *Biochim. Biophys. Acta.* **834:** 376–385.
22. Goldstein, R., O. Blondheim, E. Levy, H. Stankiewicz, and S. Freier. 1983. The fatty meal test: an alternative to stool fat analysis. *Am. J. Clin. Nutr.* **38:** 763–768.
23. Bar-On, H., E. Levy, Y. Oschry, E. Ziv, and E. Shafrir. 1984. Removal defect of very-low-density lipoproteins from diabetic rats. *Biochim. Biophys. Acta.* **793:** 115–118.
24. Lepage, G., and C. C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **27:** 114–120.
25. Bowyer, B. A., R. Fleming, D. Ilstrup, J. Nelson, S. Reek, and J. Burnes. 1986. Plasma carnitine levels in patients receiving home parenteral nutrition. *Am. J. Clin. Nutr.* **43:** 85–91.
26. Teng, B., A. Sniderman, R. M. Krauss, P. D. Kwiterovich, R. W. Milne, and Y. L. Marcel. 1985. Modulation of apoprotein B antigenic determinants in human low density lipoprotein subclasses. *J. Biol. Chem.* **260:** 5067–5072.
27. Deckelbaum, R. J., C. Dupont, J. Letarte, and P. Pencharz. 1983. Primary hypertriglyceridemia in childhood. *Am. J. Dis. Child.* **137:** 396–398.
28. Krauss, R. M., R. I. Levy, and D. S. Frederickson. 1974. Selective measurement of two lipase activities in post-heparin plasma from normal subjects and patients with hyperlipoproteinemia. *J. Clin. Invest.* **54:** 1107–1124.
29. Belfrage, P., and M. Vaughan. 1969. Simple liquid–liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. *J. Lipid Res.* **10:** 341–344.
30. Levy, E., R. Goldstein, S. Freier, and E. Shafrir. 1981. Characterization of gastric lipolytic activity. *Biochim. Biophys. Acta.* **664:** 316–326.
31. McAfee, J. G., R. G. Anse, and H. N. Wagner, Jr. 1965. Diagnostic value of scintillation scanning of the liver. *Arch. Intern. Med.* **116:** 95–110.
32. Green, M. S., G. Heiss, B. R. Rifkind, G. R. Cooper, O. D. Williams, and H. A. Tyroler. 1985. The ratio of plasma high-density lipoprotein cholesterol to total and low-density lipoprotein cholesterol: age-related changes and race and sex differences in selected North American populations. The Lipid Research Clinics Program Prevalence Study. *Circulation.* **72:** 93–104.
33. Schaefer, E. J. 1984. Clinical, biochemical, and genetic features in familial disorders of high density lipoprotein deficiency. *Arteriosclerosis.* **4:** 303–322.
34. Stanley, C. A., J. L. Mills, and L. Baker. 1981. Intragastric feeding in Type I glycogen storage disease: factors affecting the control of lactic acidemia. *Pediatr. Res.* **15:** 1504–1508.
35. Chen, Y. T., M. Cornblath, and J. B. Sidbury. 1984. Cornstarch therapy in type I glycogen storage disease. *N. Engl. J. Med.* **310:** 171–175.
36. Fernandes, J., H. Jansen, and T. C. Jansen. 1979. Nocturnal gastric drip feeding in glucose-6-phosphatase-deficient children. *Pediatr. Res.* **13:** 225–229.
37. Greene, H. L., A. E. Slonim, and I. M. Burr. 1979. Type I glycogen storage disease: a metabolic basis for advances in treatment. *Adv. Pediatr.* **26:** 63–92.
38. Frederickson, D. S., R. I. Levy, and F. T. Lindgren. 1968. A comparison of heritable abnormal lipoprotein patterns as

defined by two different techniques. *J. Clin. Invest.* **47:** 2446–2457.
39. Miller, N. E., S. N. Rao, P. Alaupovic, N. Noble, J. Slack, J. D. Brunzell, and B. Lewis. 1981. Familial apolipoprotein C-II deficiency: plasma lipoproteins and apolipoproteins in heterozygous and homozygous subjects and the effects of plasma infusion. *Eur. J. Clin. Invest.* **11:** 69–76.
40. Nikkila, E. A., M. R. Taskinen, and M. Kekki. 1978. Relation of plasma high-density lipoprotein cholesterol to lipoprotein-lipase activity in adipose tissue and skeletal muscle of man. *Atherosclerosis.* **29:** 497–501.
41. Nikkila, E. A. 1978. Metabolic and endocrine control of plasma high density lipoprotein concentration. Relation to catabolism of triglyceride-rich lipoproteins. *In* High Density Lipoprotein and Atherosclerosis. A. M. Gotto, Jr., N. E. Miller and M. F. Oliver, editors. Elsevier/North-Holland, New York. 177–192.
42. Nikkila, E. A. 1978. Metabolic regulation of plasma high density lipoprotein concentrations. *Eur. J. Clin. Invest.* **8:** 111–113.
43. Huttunen, J. K., C. Ehnholm, M. Kekki, and E. A. Nikkila. 1976. Post-heparin plasma lipoprotein lipase and hepatic lipase in normal subjects and in patients with hypertriglyceridemia: correlations to sex, age and various parameters of triglyceride metabolism. *Clin. Sci. Mol. Med.* **50:** 249–260.
44. Anderson, D. W., A. V. Nichols, S. S. Pan, and F. T. Lindgren. 1978. High density lipoprotein distribution. Resolution and determination of three major components in a normal population sample. *Atherosclerosis.* **29:** 161–179.
45. Shepherd, J., C. J. Packard, J. M. Stewart, B. D. Vallance, T. D. V. Lawrie, and H. G. Morgan. 1980. The relationship between the cholesterol content and subfraction distribution of plasma high-density lipoproteins. *Clin. Chim. Acta.* **101:** 57–62.
46. Taskinen, M. R., M. L. Kashyap, L. R. Srivastava, M. Ashraf, J. D. Johnson, G. Perisutti, D. Brady, C. J. Glueck, and R. L. Jackson. 1982. In vivo catabolism of human plasma very low density lipoproteins. Effects of VLDL concentration on the interconversion of high density lipoprotein subfractions. *Atherosclerosis.* **41:** 381–394.
47. Jonas, A., S. A. Sweeny, and P. N. Herbert. 1984. Discoidal complexes of A and C apolipoproteins with lipids and their reactions with lecithin:cholesterol acyltransferase. *J. Biol. Chem.* **259:** 6369–6375.
48. Alaupovic, P., and J. Fernandes. 1985. The serum apolipoprotein profile of patients with glucose-6-phosphatase deficiency. *Pediatr. Res.* **19:** 380–384.
49. Spooner, P. M., S. S. Chernick, M. M. Garrison, and R. O. Scow. 1979. Development of lipoprotein lipase activity and accumulation of triacylglycerol in differentiating 3T3-Li adipocytes. *J. Biol. Chem.* **254:** 1305–1311.
50. Spooner, P. M., S. S. Chernick, M. M. Garrison, and R. O. Scow. 1979. Insulin regulation of lipoprotein lipase activity and release in 3T3-Li adipocytes. *J. Biol. Chem.* **254:** 10021–10029.
51. Blackard, W. G., and C. N. Aprill. 1967. Mechanisms of action of diazoxide. *J. Lab. Clin. Med.* **69:** 960–967.
52. Wolff, F. W., R. G. Langdon, B. H. Ruebner, C. Hollander, and R. D. Skoglund. 1963. A new form of experimental diabetes. *Diabetes.* **12:** 335–338.
53. Baker, L., R. Kaye, A. W. Root, and A. L. N. Prasad. 1967. Diazoxide treatment of idiopathic hypoglycemia of infancy. *J. Pediatr.* **71:** 494–505.
54. Stanley, C. A., and L. Baker. 1976. Hyperinsulinism in infants and children: diagnosis and therapy. *Pediatr. Res.* **23:** 315–325.
55. Spergel, G., and S. J. Bleicher. 1966. Effects of diazoxide administration on plasma glucose, insulin, and lipids in von Gierke's disease. *Diabetes.* **15:** 406–410.
56. Rennert, O. M., and D. Mukhopadhyay. 1968. Diazoxide in Von Gierke's disease. *Arch. Dis. Child.* **43:** 358–361.
57. Eaton, R. P., and W. H. Nye. 1973. The relation between insulin secretion and triglyceride concentration in endogenous lipemia. *J. Lab. Clin. Med.* **81:** 682–695.
58. Zarday, A., J. Victoria, and F. Wolff. 1966. The effect of diazoxide on catecholamines. *Metabolism.* **15:** 257–261.
59. Chait, A., E. Janus, A. S. Mason, and B. Lewis. 1979. Lipodystrophy with hyperlipidaemia: the role of insulin in very low density lipoprotein over-synthesis. *Clin. Endocrinol.* **10:** 173–178.
60. Carlson, L. A., and D. Ballantyne. 1976. Changing relative proportion of apolipoprotein CII and CIII of very low density lipoproteins in hypertriglyceridemia. *Atherosclerosis.* **23:** 563–568.
61. Boberg, J., M. Boberg, R. Gross, S. Grundy, J. Augustin, and V. Brown. 1977. The effect of treatment with clofibrate on hepatic triglyceride and lipoprotein lipase activities on postheparin plasma in male patients with hyperlipoproteinemia. *Atherosclerosis.* **27:** 499–503.
62. Curry, M. D., W. J. McConathy, J. D. Fesmire, and P. Alaupovic. 1981. Quantitative determination of apolipoproteins C-I and C-II in human plasma by separate electroimmunoassays. *Clin. Chem.* **27:** 543–548.
63. Nikkila, E. A., J. K. Huttunen, and C. Ehnholm. 1977. Effect of clofibrate on postheparin plasma triglyceride lipase activities in patients with hypertriglyceridemia. *Metabolism.* **26:** 179–186.
64. Quinn, D., K. Shirai, and R. L. Jackson. 1982. Lipoprotein lipase: mechanism of action and role in lipoprotein metabolism. *Prog. Lipid Res.* **22:** 35–78.
65. Grundy, S. M. 1984. Pathogenesis of hyperlipoproteinemia. *J. Lipid Res.* **25:** 1611–1618.
66. Vergroesen, A. J. 1985. Effects of dietary lipid composition on serum lipoproteins. *Prog. Clin. Biol. Res.* **188:** 161–167.