

A-I-Milano apoprotein. Decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family.

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Research Article

Significant hypertriglyceridemia with a very marked decrease of high density lipoproteins (HDL)-cholesterol levels (7-14 mg/dl) was detected in three members (father, son, and daughter) of an Italian family. The three affected individuals did not show any clinical signs of atherosclerosis, nor was the atherosclerotic disease significantly present in the family. Lipoprotein lipase and lecithin:cholesterol acyltransferase activities were normal or slightly reduced. Morphological and compositional studies of HDL in the subjects showed a significant enlargement of the lipoprotein particles (approximately 120 vs. approximately 94 Å for control HDL) and a concomitant increase in the triglyceride content. Analytical isoelectric focusing of HDL apoproteins provided evidence for multiple isoproteins in the apoprotein(apo)-A-I range, with nine different bands being detected instead of the usual four bands observed in normal subjects. Two-dimensional immunoelectrophoresis against apo-A antiserum indicated a clear reduction of apo-A in the alpha electrophoretic region, with splitting of the protein "peak." The observation in otherwise clinically healthy subjects of hypertriglyceridemia, reduced HDL-cholesterol, and marked apoprotein abnormalities, without a significant incidence of atherosclerotic disease in the family suggests this is a new disease entity in the field of lipoprotein pathology, very probably related to an altered amino acid composition of the apo-A-I protein (see Weisgraber et al. 1980. *J. Clin. Invest.* 66: 901-907).

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A-I_{Milano} Apoprotein

DECREASED HIGH DENSITY LIPOPROTEIN CHOLESTEROL LEVELS WITH SIGNIFICANT LIPOPROTEIN MODIFICATIONS AND WITHOUT CLINICAL ATHEROSCLEROSIS IN AN ITALIAN FAMILY

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ABSTRACT Significant hypertriglyceridemia with a very marked decrease of high density lipoproteins (HDL)-cholesterol levels (7–14 mg/dl) was detected in three members (father, son, and daughter) of an Italian family. The three affected individuals did not show any clinical signs of atherosclerosis, nor was the atherosclerotic disease significantly present in the family. Lipoprotein lipase and lecithin:cholesterol acyltransferase activities were normal or slightly reduced. Morphological and compositional studies of HDL in the subjects showed a significant enlargement of the lipoprotein particles (~120 vs. ~94 Å for control HDL) and a concomitant increase in the triglyceride content. Analytical isoelectric focusing of HDL apoproteins provided evidence for multiple isoproteins in the apoprotein(apo)-A-I range, with nine different bands being detected instead of the usual four bands observed in normal subjects. Two-dimensional immunoelectrophoresis against apo-A antiserum indicated a clear reduction of apo-A in the alpha electrophoretic region, with splitting of the protein “peak.” The observation in otherwise clinically healthy subjects of hypertriglyceridemia, reduced HDL-cholesterol, and marked apoprotein abnormalities, without a significant incidence of atherosclerotic disease in the family suggests this is a new disease entity in the field of lipoprotein pathology, very probably related to an altered amino acid composition of the apo-A-I protein (see Weisgraber et al. 1980. *J. Clin. Invest.* 66: 901–907).

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INTRODUCTION

Plasma levels of high density lipoproteins (HDL)¹ and, in particular, of HDL-cholesterol, have received increasing attention in the past few years because of the negative statistical correlation between HDL-cholesterol levels and the incidence of ischemic vascular diseases, both of the heart (1, 2) and of the central nervous system (3). Inversely, a protective effect has been postulated for increased HDL levels, on the assumption that in this condition an increased cholesterol removal from tissues (4, 5) and/or a decreased delivery of cholesterol by the “low density lipoprotein receptors” (6) may occur.

At the two extremes of HDL-cholesterol distribution, a striking difference in the risk of clinical atherosclerosis is found; in particular, subjects with markedly elevated HDL-cholesterol may have a “longevity syndrome” (7), whereas a familial pattern of decreased HDL-cholesterol may be associated with a high incidence of myocardial infarction, particularly when hypercholesterolemia is also present (8). Decreased HDL-cholesterol levels are frequently found in patients with hypertriglyceridemia (Fredrickson types I, IV, and V), where a negative correlation has been suggested (9) but not generally confirmed (10) between triglyceride levels in very low density lipoproteins (VLDL) and HDL-cholesterol. A decrease in the C-II peptide activation of lipoprotein lipase is, in this case, suggested as the mechanism of hypertriglyceridemia (11).

Patients with a complete or almost complete absence

¹ *Abbreviations used in this paper:* HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; LPL, lipoprotein lipase; TG, plasma triglycerides; VLDL, very low density lipoproteins.

of HDL are diagnosed as having Tangier disease (12). In this disease, the total absence of HDL and HDL-cholesterol is usually accompanied by hypolipidemia, multiple lipoprotein abnormalities (13, 14), and at times deficient activities of lipoprotein lipase and lecithin: cholesterol acyltransferase (15). Characteristic clinical symptoms (enlarged tonsils, splenomegaly) are secondary to cholesterol accumulation in tissues (16). In Tangier disease, a defective assembly of HDL is postulated, based on a normal presence of apoprotein (apo)-A-I (the major protein component of HDL) in the mucosal cells of the intestinal wall (17) and on the detection of apo-A-I in the infranant proteins after ultracentrifugation of plasma lipoproteins (18). Furthermore, a structural abnormality of A-I has been proposed as the defect in Tangier disease (18). Contrary to expectations, in Tangier disease clinical atherosclerosis is not found, possibly because cholesterol in the tissues removed by nascent HDL is not deposited in arterial smooth muscle cells but only in tissue macrophages (19).

The clinical observation of a family with hypertriglyceridemia and a very severe reduction of plasma HDL, HDL-cholesterol, and apo-A-I levels, without clinical findings of Tangier disease or clinical atherosclerosis, prompted more detailed clinical and biochemical investigations of this syndrome. Analysis of HDL apoproteins provided evidence that this is the first human disease characterized by an abnormal amino acid composition of an apolipoprotein, as will be described in the accompanying report (20).

METHODS

Patients. The family, D., consists of a father and three children—a boy and two girls—of the respective ages in 1980 of 49, 19, 13, and 12. The father, D.V., was referred to the Lipid Center in Milan in 1975 because of moderate hypertriglyceridemia (total plasma triglyceride levels between 300 and 400 mg/dl) apparently resistant to diet and clofibrate treatment. The patient's private physician indicated that clofibrate treatment was poorly tolerated and that it appeared to induce a marked increase of plasma triglyceride (TG) levels. This finding could not be confirmed because the patient refused to repeat this medication.

At the first clinic visit (6 May 1975), standard lipid and lipoprotein analysis indicated a moderate hypertriglyceridemia (244 mg/dl) with a normal total plasma cholesterol (220 mg/dl). On agarose electrophoresis, a wide, heavily stained pre-beta band and an almost complete absence of an alpha lipoprotein band were observed. The HDL-cholesterol level, determined at this visit by selective precipitation of very low and low density lipoproteins (21), was 12 mg/dl (Fig. 1).

Historically, the patient D.V. complained only of irregular bowel habits, markedly influenced by psychological conditions. This had been diagnosed as "irritable colon." He had undergone several tonsillectomies (at ages 3, 30, and 32), apparently for repeated streptococcal infections. The ear-nose-throat specialists had not noted any unusual characteristics of the tissue after tonsillectomy. The patient reported

numerous episodes of lymphadenopathies and bronchitis. At age 36 he had undergone total gastrectomy because of a bleeding duodenal ulcer. Subsequently, bowel complaints were frequent.

The patient denied any symptoms indicating coronary or peripheral artery disease. Coronary disease was described only in one cousin, and diabetes mellitus, in a brother. The parents of the subject are alive. The mother, age 73, has suffered a stroke, but otherwise both parents are healthy. The father is 79.

The physical examination of the patient disclosed a normally developed, slightly overweight (166 cm, 75 kg) white male. No abdominal masses could be palpated. Eye grounds and EKG were normal, as were the standard biochemical tests and urinalysis.

In the years 1975–78, diet and drug treatment were administered to lower the markedly elevated TG levels of the patient. Polyenyl phospholipids (600 mg/d) (22) (Lipostabil, Nattermann, German Federal Republic) were given at the suggestion of Dr. H. Peeters (Brussels, Belgium). Metformin treatment (Glucophage, Spemsa, Florence, Italy) was also administered (23). Based on recent experimental and clinical evidence (17, 24) suggesting a role for the intestine in apoprotein A-I synthesis, a diet markedly enriched in fat (~55–60% of calories) was also recently prescribed.

The wife and children of the subject were also examined for lipid and lipoprotein levels and for physical signs. The wife, of German origin, had a normal lipid and lipoprotein profile. The boy, D.M., and the younger girl, D.A., examined at ages 15 and 9, were also found to have markedly decreased HDL-cholesterol levels. In contrast, the other daughter, D.E., age 10, had a normal HDL-cholesterol level (42 mg/dl) and no other lipid abnormalities. She was later found to have none of the typical apoprotein changes detected in the father and in her brother and sister (see below). Therefore, she was not studied further, except for a detailed investigation of plasma lipoprotein composition.

The two children with decreased HDL levels also had slightly elevated TG levels. In the boy, a type IV electrophoretic profile was detected. Both children have undergone tonsillectomies; the ear-nose-throat specialist stated that the removed tonsils showed only signs of infection. Neither of the two has any significant physical signs and hepatosplenomegaly was absent. Biochemical tests disclosed only an elevation of alkaline phosphatase in the girl (403 mU/ml vs. normal values of 60–170 mU/ml). This finding could not be attributed to any specific disease or syndrome. No attempts were made to lower TG in the boy, except to suggest a moderately reduced carbohydrate diet. In the 5 yr these two children have been observed in our center, their HDL-cholesterol levels have not changed significantly, and they have shown a normal rate of physical and intellectual growth.

Preparation of lipoproteins and apoproteins. Ultracentrifugal isolation of lipoproteins was carried out according to the procedure described by Havel et al. (25). For routine follow-up of the patients, the National Institutes of Health guidelines (26) were applied, i.e., ultracentrifugation of plasma at $d < 1.006$ and precipitation of low density lipoproteins (LDL) in the infranate by heparin-MnCl₂ (20). When HDL were isolated by ultracentrifugation, they were subjected to only one ultracentrifugal washing, due to the paucity of the available material.

Lipoprotein fractions were exhaustively dialyzed against 0.15 M NaCl, 1 mM EDTA (pH 7.4). Samples were delipidated with ethanol:diethyl ether (3:1 vol/vol) according to Brown et al. (27).

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis of isolated apoproteins was carried out by two

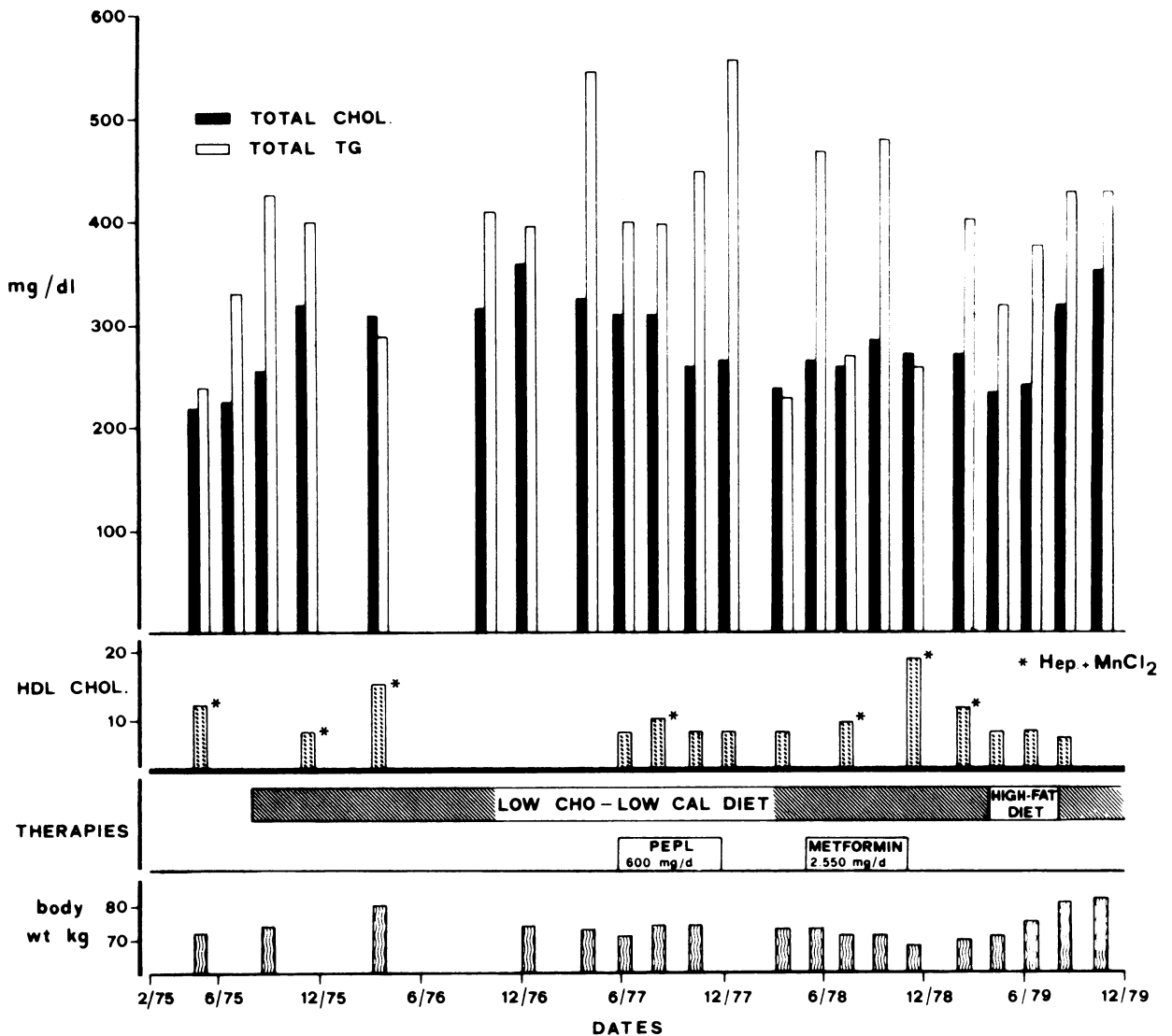


FIGURE 1 Clinical course of patient D.V. Plasma cholesterol and TG, HDL-cholesterol and body weight. A relative stability of the HDL-cholesterol levels, in spite of the drug and diet treatments, is noted, particularly when the ultracentrifuge determinations (without asterisks) are considered. Polyenyl phospholipids (PEPL) and metformin did not appear to exert any significant effect on plasma lipid and lipoproteins. Notice the tendency of total plasma cholesterol levels to increase as body weight rises.

techniques, viz., sodium dodecyl sulfate (SDS) and urea gel electrophoresis. For SDS-polyacrylamide gel electrophoresis, the procedure described by Weber and Osborn (28) was followed using 10% acrylamide gels containing 0.1% SDS. 5% mercaptoethanol was added as a reducing agent only in specified samples. Molecular weights were determined from recorded scans by comparison with added molecular weight markers and with the location of apoproteins of known molecular weights. The procedure for the urea gel electrophoretic separation followed the methodology described by Davis (29), using 7.5 and 10% acrylamide gels containing 8.0 M urea.

Isoelectric focusing. Polyacrylamide gel isoelectric focusing was carried out at different pH ranges after apoprotein

solubilization in 100 μ l 8.0 M urea and 20 μ l 40% sucrose (wt/vol). In specified samples, 5 μ l of mercaptoethanol was also added to the system. 7.5% polyacrylamide gels containing 8.0 M urea and 2% Ampholine (LKB Produkter, Bromma, Sweden) were used for the pH ranges 3.5–10.0 and 4.0–6.0. The sample (50–75 μ g of proteins) was applied to the top of each gel (0.5 \times 9 cm) and overlaid with the upper electrolyte (0.02 M NaOH), the upper chamber being filled with the solution. The lower electrolyte was 0.01 M H_3PO_4 . The gels were stained according to the method of Malik and Berrie (30). A blank gel was included for the determination of the pH profiles.

Enzyme activity determination. Lipoprotein lipase (LPL) activity was determined in the three subjects by intravenous

injection of 100 IU/kg sodium-heparin (Liquemin, Roche Diagnostics Div., Hoffman-La Roche Inc., Nutley, N. J.). Plasma samples were drawn after 5 and 45 min, and the lipolytic activity determined on Intralipid (Vitrum AB, Stockholm, Sweden) substrate, as suggested by Boberg (31). This technique was recently shown to assess the LPL activity accurately without being affected by the hepatic lipase activity (32).

Lecithin:cholesterol acyltransferase (LCAT) was tested in the three subjects by the nonisotopic method described by Patsch et al. (33). A decrease in plasma free cholesterol concentration was measured by enzyme methodology (Boehringer Biochima, German Federal Republic), before and after incubation at 37°C.

Immunological methods. Immunological analyses of plasma apo-B and apo-A-I were carried out with monospecific antisera raised in rabbits. The apo-B content of our samples was measured by radial immunodiffusion, using commercial immunoplates (M-Partigen, beta lipoprotein, Behringwerke AG, Marburg-Lahn, German Federal Republic). As an apo-B reference standard, two preparations were used: a standard serum, purchased from Behringwerke, and an LDL preparation (fraction $d = 1.030-1.050$), previously tested against anti-apo-B, apo-A-I, apo-CII, apo-CIII, albumin, and total immunoglobulins, to demonstrate the presence of protein contaminants other than apo-B.

The apo-A-I content of native serum lipoprotein fractions and of the $d > 1.210$ bottom fraction were measured by electroimmunodiffusion (34). Two preparations were used as standards, including a freshly prepared pool of 100 normolipemic subjects and a preparation of pure apo-A-I obtained by column chromatography (18).

A two-dimensional immunoelectrophoretic analysis was also carried out on whole plasma, which was reacted against anti-apo-A (A-I and A-II) and anti-apo-B. After the two-dimensional immunoelectrophoretic run, the templates were stained for lipids and proteins, using Fat Red 7B and Coomassie Brilliant Blue R-250 solutions, respectively.

Other methods. Plasma cholesterol and TG levels were determined by standard automated colorimetric procedures after isopropanol extraction (35, 36). Phospholipids were determined according to Rouser et al. (37).

Electrophoretic separation of plasma lipoproteins was carried out on agarose gels (38) and on polyacrylamide gels (39). These techniques were also applied to isolated lipoproteins.

Electron microscopy of ultracentrifugally isolated HDL from patient D.V. and from a normal subject was performed after extensive dialysis, as reported above for apoprotein studies. Lipoprotein particles were negatively stained with 0.1 M potassium phosphotungstate (40) and examined on a Philips EM 200 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. J.).

All of the biochemical tests were compared with normal values collected in our laboratory and with data reported in the literature.

RESULTS

Plasma lipid and lipoprotein findings. Both the father and son from years 1975 to 79 constantly exhibited elevated TG levels with a type IV lipoprotein profile (Table I). The affected daughter (D.A.) had normal plasma lipid levels initially (1976: cholesterol, 175 mg/dl; TG, 140 mg/dl), but more recently she, too, has shown an elevation of TG levels (Table I). On the

TABLE I
Plasma and Lipoprotein Lipids in the D. Family

	D.V. father 49 yr	D.M. son 19 yr	D.A. daughter 12 yr	D.E. daughter 13 yr
	mg/dl			
Plasma				
Total cholesterol	231.4	190.3	183.9	216.2
Esterified cholesterol	160.8	135.0	131.3	158.6
Free cholesterol	70.6	55.3	52.6	57.6
Triglycerides	318.8	272.6	180.6	116.6
Phospholipids	280.0	125.3	115.6	80.8
Apo-A-I	35.2	50.2	73.6	116.5
Apo-B	127.0	97.7	94.2	96.3
VLDL ($d < 1.006$)				
Total cholesterol	40.8	26.6	11.5	15.6
Esterified cholesterol	21.1	11.4	5.8	7.1
Free cholesterol	19.7	15.2	5.7	8.5
Triglycerides	206.0	189.0	102.0	73.8
LDL ($d = 1.006-1.063$)				
Total cholesterol	183.9	156.3	154.8	156.3
Esterified cholesterol	134.6	117.9	112.7	112.0
Free cholesterol	49.3	38.4	42.1	44.3
Triglycerides	105.0	75.0	67.0	25.0
HDL ($d = 1.063-1.21$)				
Total cholesterol	6.7	7.4	17.6	44.3
Esterified cholesterol	5.1	5.7	12.8	38.2
Free cholesterol	1.6	1.7	4.8	6.1
Triglycerides	7.3	7.6	10.6	17.8
Phospholipids	22.0	23.1	36.1	52.8
Proteins	27.8	37.7	63.3	142.4

All samples were collected in May 1979.

other hand, all three affected subjects showed a marked reduction of plasma HDL-cholesterol levels, as determined by ultracentrifugation and selective precipitation of VLDL and LDL.

Table I reports compositional studies of plasma lipoproteins in the three affected subjects and in the unaffected daughter (D.E.). In every affected subject, an elevation of TG was noted in all major lipoprotein classes. The compositional modification of HDL, with marked TG enrichment, suggested that these particles may be enlarged. This has been confirmed by electron microscopy of the isolated lipoproteins (Fig. 2).

Apoprotein B levels were within normal limits in all subjects. In contrast, total plasma apo-A-I was markedly decreased in both father and son (<50% of normal), and less so in the daughter (~70% of normal) (Table I). Most of apo-A-I was found in HDL in all three subjects. No immunoassayable apoprotein could be detected in the $d > 1.21$ fraction.

Diet and drug studies in patient D.V. During the years of follow-up, several attempts have been made to

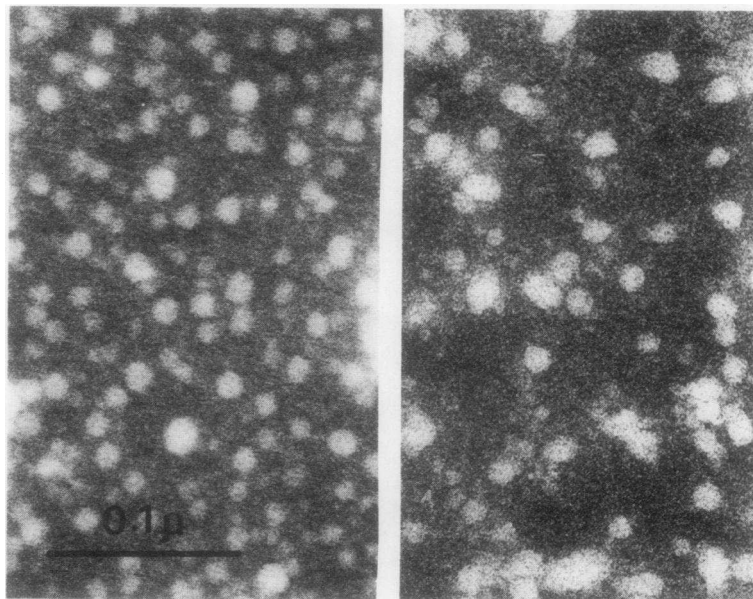


FIGURE 2 Electron micrographs of negatively stained HDL particles from the plasma of patient D.V. and from a normal subject. Particles from the patient are larger (mean \pm SD of 250 particles: control, 94 ± 17 Å; patient, 120 ± 25 Å; $P < 0.001$) and irregular in shape. $\times 260,000$.

lower the elevated plasma lipid levels of patient D.V. (Fig. 1). In 1976, for as yet unexplained reasons (possibly because the diet had been restricted in calories and increased in the percentage of fat), the patient had a rise of total plasma cholesterol, up to 362 mg/dl, in spite of insignificant changes in total TG and HDL-cholesterol levels. At this time, he was started on polyenyl phospholipids (600 mg/d) (22). The treatment did not exert any significant effect, with the possible exception of a slight reduction of total cholesterol levels. HDL-cholesterol levels were not changed.

After this treatment, the patient was placed on metformin (23) (2,550 mg/d). He tolerated the treatment poorly, suffering diarrhea and discomfort at the gastric stump. This was probably the cause of a significant weight loss (~ 5 kg) during the 5-mo course of

treatment. No significant TG reduction was noted, although total cholesterol levels were reduced. At the end of the metformin treatment, HDL-cholesterol levels were possibly raised (19 mg/dl), as tested by selective precipitation. The treatment was, however, not continued.

More recently, the patient has been given a fat-enriched diet, on the assumption that this might increase HDL protein synthesis by the intestine (17, 24). This treatment resulted only in some increase of total TG and no changes in plasma cholesterol or HDL-cholesterol levels (Table II).

LPL and LCAT activities. Enzyme activities, as determined in all three subjects, were within normal limits. LPL, measured only from nonhepatic sources, was only slightly decreased in the son (just below the

TABLE II
Plasma and Lipoprotein Lipids in Patient D.V. before and after 2 Mo
(June, July 1979) on a Fat-enriched Diet

	Before		After	
	Cholesterol	Plasma triglyceride	Cholesterol	Plasma triglyceride
	mg/dl		mg/dl	
Plasma	289	350	286	398
VLDL ($d < 1.006$)	41	228	44	282
LDL ($d = 1.006-1.063$)	241	114	235	107
HDL ($d = 1.063-1.21$)	7	8	7	9

TABLE III
Postheparin LPL and Lecithin: LCAT Activities in Three Members of the D. Family Affected with Lipoprotein Disorder

	D.V.	D.M.	D.A.	Healthy controls
LPL ($\mu\text{mol/ml/h}$) (Sept. 1976)	7.07 \pm 0.1*	5.55 \pm 0.4	9.23 \pm 0.3	7.11 \pm 1.10†
LCAT (nmol/ml/h) (May 1979)	135.80 \pm 21.3	86.80 \pm 21.6	103.70 \pm 19.2	91 \pm 15 (males)§ 62 \pm 12 (females)§

* Values are mean \pm SD; $n = 3$.

† Reference 32.

§ Reference 33.

normal limits of our laboratory) (Table III). Similarly, LCAT activities were normal in the father and daughter, and somewhat reduced in the son (Table III).

Electrophoretic separation of lipoproteins and apoproteins. Electrophoretic separation of plasma lipoproteins on polyacrylamide gels (Fig. 3) showed that all three affected subjects had a marked decrease of lipoproteins with the characteristic migration of HDL. In both father and son, there was a noticeably increased staining of the band corresponding to VLDL. The electrophoretic mobility of HDL, separated and concentrated by ultracentrifugation in patient D.V., was compared with that of similarly isolated lipoproteins from a normal subject. Fig. 4 shows that the mobility of the two lipoproteins was the same.

The most remarkable observations in the HDL apoproteins were obtained with the polyacrylamide gel isoelectric focusing procedure. More detailed information on the results with SDS-polyacrylamide gel electrophoresis will be given in the accompanying report (20). Polyacrylamide gel isoelectric focusing patterns showed nine different bands at the pI interval corresponding to apo-A-I (5.04–5.52), instead of the

usual four isoproteins detected in normal subjects (Fig. 5). Moreover, the most alkaline band of normal apo-A-I was practically absent, whereas more bands were evident in the pI range corresponding to the other three isoproteins of apo-A-I. After mercaptoethanol reduction, the apo-A-I still did not acquire a normal pattern.

Immunological tests. Two-dimensional immunoelectrophoresis was performed on plasma lipoproteins from normal subjects and from the three affected subjects with antisera against apo-A and apo-B. After staining for both lipid and proteins (Fig. 6), the pattern clearly indicated a marked reduction of apo-A in the alpha electrophoretic region. Moreover, the very low protein "peak" in this region appeared to be split, due to the separation of apo-A-I (faster) from apo-A-II (slower) (18).

DISCUSSION

The combination of (a) marked familial decrease of HDL-cholesterol and of apo-A-I, (b) hypertriglyceridemia that is resistant to diet and drug treatments, (c) changes of the HDL composition and apoprotein pattern, (d) normal LPL and LCAT activities, and (e) the

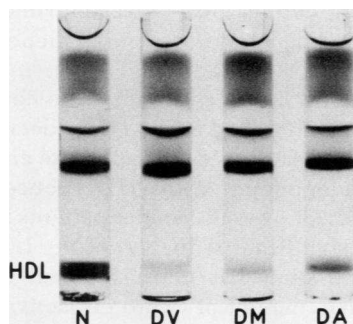


FIGURE 3 Polyacrylamide gel electrophoretic pattern of plasma lipoproteins from affected members of the D. family and from a normal subject. A markedly decreased staining is noted for particles migrating in the HDL range, particularly in the father (D.V.) and son (D.M.), and less so in the daughter (D.A.).

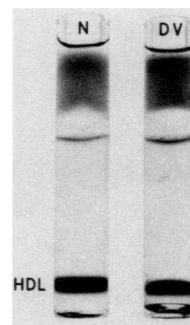


FIGURE 4 Electrophoretic mobility of isolated HDL from a normal subject (left) and from patient D.V. (right). A similar amount of lipoprotein lipids was applied to each gel. No significant differences in mobility are noted between the two lipoproteins.

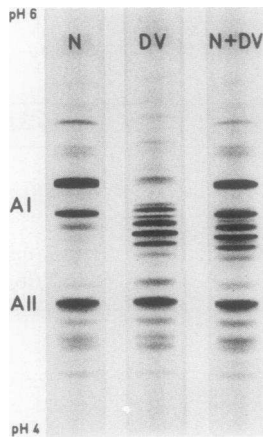


FIGURE 5 Isoelectric focusing patterns (pH range 4.0–6.0) of isolated HDL from a normal subject, from patient D.V., and from a mixture of the two lipoproteins. Nine different bands are evident in the D.V. lipoproteins in the pI range of apo-A-I (pH 5.04–5.52) in contrast to the four normal isoprotein bands. No mercaptoethanol was added to these samples.

absence both of a significant increase of atherosclerotic vascular disease in the family and of cholesterol accumulation in tissues strongly suggests that this is a new disease entity in the field of lipoprotein pathology. This new disease is of interest because it combines a decrease of HDL lipids and apoproteins with other derangements of lipoprotein metabolism, in particular

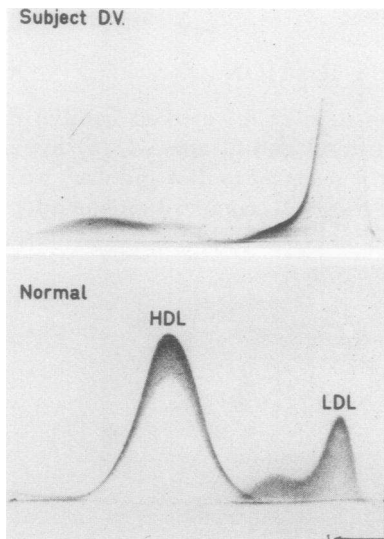


FIGURE 6 Two-dimensional immunoelectrophoretic patterns of plasma lipoproteins from a normal subject and from patient D.V. The separated lipoproteins were reacted against anti-apo-B and anti-apo-A antibodies. Patient D.V. shows clear evidence of increased B apoprotein in the beta, pre-beta region, in contrast to the normal subject. More strikingly, the alpha-migrating lipoproteins are markedly decreased in the patient, with splitting of the protein peak.

hypertriglyceridemia, without clinical symptoms common to other lipid disorders.

Hypertriglyceridemia, the major complaint for which patient D.V. was referred to our center, was resistant to diet and drug treatment. Calorie and carbohydrate reduction possibly induced a rise of total plasma cholesterol, whereas drug treatments (polyenyl phospholipids or metformin) produced inconsistent results. With metformin there was perhaps some increase of HDL-cholesterol, but the poor tolerance of this drug by the gastrectomized patient required that treatment be discontinued. The historical evidence that clofibrate treatment also was ineffective and may have induced a TG rise suggests that even activation of the catabolic pathway for VLDL, the postulated mechanism of action of this hypolipidemic drug (41), did not result in any significant therapeutic effect. We were unable to confirm this ourselves because the patient declined to repeat the clofibrate therapy.

The apparent analogies between this condition and Tangier disease prompted historical investigations of the tonsillectomies and a careful search for hepatosplenomegaly. Tonsillectomies in the subjects were apparently related to streptococcal infections. Hepatosplenomegaly was not detected. Two-dimensional immunoelectrophoretic studies of plasma lipoproteins were also carried out to distinguish this syndrome from Tangier disease (18). The immunoelectrophoretic patterns, carried out under the same conditions as followed for Tangier disease (18), showed significant differences. In particular, a splitting of the alpha-moving band was noted with anti-apo-A serum. Moreover, a larger amount of immunoreactive apo-A-I was present in both serum and HDL than previously observed in Tangier patients. Finally, no apo-A-I was detected in the infranant fraction of ultracentrifugally separated lipoproteins. Another disorder characterized by reduced plasma HDL levels and increased triglyceride levels is "fish-eye" disease (42). The major clinical feature is severe corneal opacity accompanied by visual impairment. Our patients had no evidence of corneal opacities.

That LPL and LCAT activities were normal in both father and daughter, and only slightly decreased in the son, indicated that the activities of these enzymes were relatively independent of the HDL concentrations in these patients. Studies of Tangier patients (15, 43) have indicated a significant reduction of the LCAT activity and only one study has found reduced LPL activity (15). The total amount of C protein activators of LPL may have been low in HDL, but it was normal in the VLDL of these patients (unpublished observation). On the other hand, the normal LCAT activity in the absence of a significant tissue cholesterol deposition points to an efficient removal mechanism of tissue cholesterol. Either effective catalytic properties of the

markedly diminished apo-A-I and/or a rapid but functional turnover may explain the lack of cholesterol deposition in our patients. This would contrast with the case of Tangier HDL, where apo-A-I has an accelerated turnover (44), but does not efficiently remove cholesterol from tissue.

The presented clinical observations raise several questions. The first is related to the significant hypertriglyceridemia in some of the subjects. The negative correlation between HDL levels and VLDL plasma triglycerides (9), not confirmed by all authors (10), has been explained based on a deficiency of LPL activators in severely hypertriglyceridemic patients (11). In this new disease, C apoproteins were not studied in detail; however, they represented a normal percentage of total apoproteins both in VLDL and HDL.

Studies already in progress on this new disease include the preparation of the pedigree of the family, because it appears that the disease has a very strong penetration. The presence of low HDL and an abnormal A-I apoprotein (20) in this family may make possible a more complete understanding of the physiologic and metabolic role of HDL, especially in view of the postulated protective role of this lipoprotein fraction against clinical atherosclerosis.

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