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Serum Lipoprotein Concentration and Composition in Healthy 50-year-old Men

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ABSTRACT

The classification of hyperlipoproteinaemias (HLP) is based on arbitrary limits between "normolipidaemia" and "hyperlipidaemia". 92 randomly selected healthy 50-year-old men were studied regarding serum lipoprotein (LP) lipid composition to define limits for "normality". Cholesterol and triglyceride concentrations were determined and the individual ratios between cholesterol and triglycerides were calculated in the ultracentrifugally isolated LP density classes. A significant linear correlation between cholesterol and triglyceride concentrations was found in the very low density lipoproteins (VLDL) and low density lipoproteins (LDL) but not in the high density lipoproteins (HDL). When different percentile limits for LDL cholesterol and VLDL triglycerides were used as cut-off points for "normality" not only the absolute prevalence but also the relative frequency of different types of HLP was influenced. 26 overweight men studied separately showed significantly increased VLDL cholesterol and triglyceride levels with maintenance of the normal ratio cholesterol/triglycerides of this LP class compared with non-obese subjects. Three different kinds of "extra bands" were occasionally seen on agarose electrophoresis: a "double pre-beta" band in whole serum (3%), a "sinking pre-beta" band with density >1.006 (17%) and a "late pre-beta" band in VLDL (22%). While "sinking pre-beta" is identical with Lp (*a*) LP the "late pre-beta" band probably represents a certain accumulation of "intermediary particles".

INTRODUCTION

Several risk factors for development of premature ischaemic heart disease (IHD) have been identified including elevated cholesterol (1, 24) and triglyceride (11) levels in serum. Serum lipoprotein (LP) levels vary considerably between different populations because of genetic and environmental factors. The classification system for hyperlipoproteinaemias (HLP) suggested by Fredrickson et al. (16, 17) has been generally accepted and is used in reports from all parts of the world concerning the prevalence of LP abnormalities in different populations. This system is based on a definition of limits between "normal" and "elevated" LP levels. The setting of these limits is arbitrary and will differ between communities because of regional differences in LP levels and possibly also because of methodological reasons. "Normal" limits thus have to be established for each community. "Normality" is not necessarily identical with desirable levels.

Several population studies from different countries regarding cholesterol and/or triglyceride levels in serum have been published (e.g. 9, 23, 14, 36, 25, 42, 19, 18, 27, 12). Only few studies, however, have dealt with the "normal" lipid distribution of and the relation between cholesterol and triglycerides in serum LP of different density classes.

HLP may be due to raised lipid content of any of the three major LP classes, the very low (VLDL), the low (LDL) and the high (HDL) density LP alone or in combination. Knowledge of "normal" ranges for lipid levels other than cholesterol in LDL and triglycerides in VLDL is sometimes essential for a correct diagnosis. An example is HLP type III where knowledge of "normal" ratios is of prime importance for the diagnosis as it is characterized by a change in cholesterol/triglyceride ratio in VLDL (21) and LDL (41) although the total lipid values may be within the normal range.

In order to get a detailed information on the variations in LP lipid composition we have performed LP analyses in a random sample of apparently healthy 50-year-old men thus avoiding the influence of sex and age. Lipid levels in the different LP density classes were determined after preparative ultracentrifugation. The frequency of atypical LP pattern on agarose electrophoresis was re-

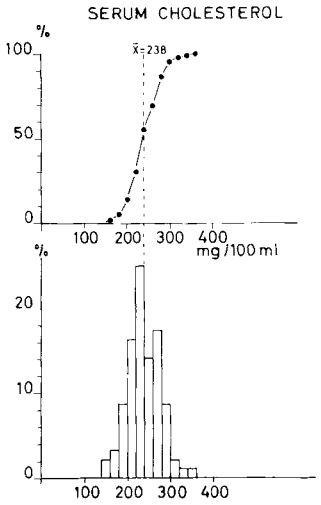

Fig. 1. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of serum cholesterol in apparently healthy 50-year-old men in Uppsala.

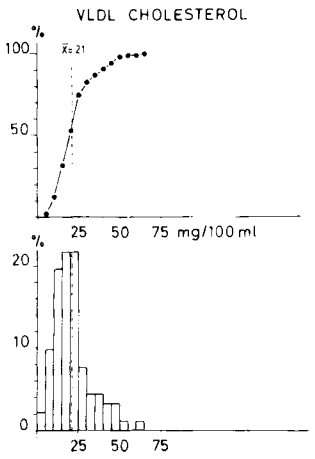

Fig. 3. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of VLDL cholesterol in apparently healthy 50-year-old men in Uppsala.

corded. Based on the study "normal" male limits for cholesterol and triglycerides in the LP classes as well as for the relative concentration between these lipids were suggested.

MATERIAL AND METHODS

From September 1970 to September 1973 a health examination survey was offered all men living in the city of Uppsala, Sweden, born in 1920–24. The examination had the special aim of identifying risk factors for IHD (22) and was carried out at the outpatient clinic at the Department of Medicine at the University hospital. A total number of 2322 men were examined and the participation rate was 83.9%.

During this investigation 4–5 men, aged 50, were randomly selected each week for serum LP analysis which was performed at the Department of Geriatrics, University of Uppsala, from November 1971 to June 1972. By extending the sampling over a period of eight months the seasonal variations (14) were minimized. The numbers selected were due to availability of holes in the rotor at the LP laboratory. This number was reported to the clinic once a week and blood samples were drawn from consecutive subjects on the morning of the next day. A total number of 131 men were sampled. The investigation started at 7.30 a.m. and blood samples were taken after 10 minutes' rest.

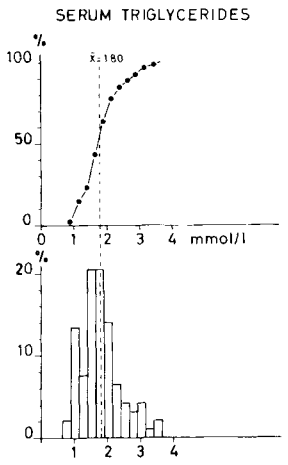

Fig. 2. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of serum triglycerides in apparently healthy 50-year-old men in Uppsala.

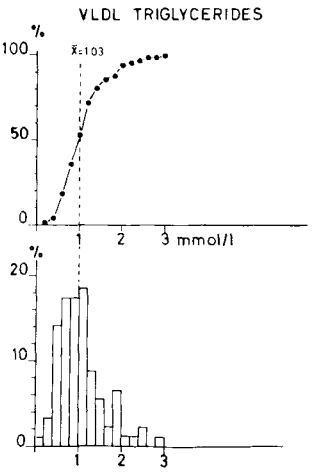

Fig. 4. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of VLDL triglycerides in apparently healthy 50-year-old men in Uppsala.

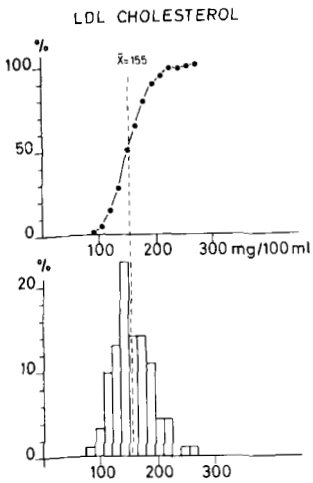

Fig. 5. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of LDL cholesterol in apparently healthy 50-year-old men in Uppsala.

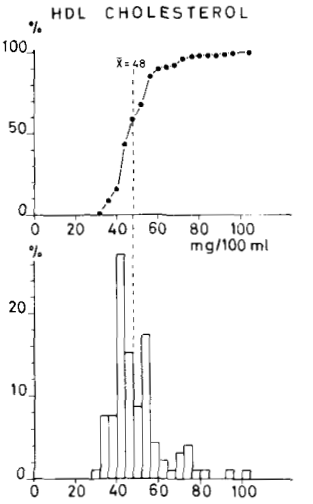

Fig. 7. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of HDL cholesterol in apparently healthy 50-year-old men in Uppsala.

LP analysis

Blood samples were drawn by venipuncture after overnight fast without venostasis. The men were asked not to smoke after midnight. The blood was allowed to stand for 2 hours at room temperature for clot retraction. After separation of serum by low speed centrifugation sodium EDTA (5%) was added to a final concentration of 0.05% and the samples were stored at 4°C. Separation of LP classes by preparative ultracentrifugation according to Havel et al. (20) was begun on the same day. The detailed procedure has been described elsewhere (7). In essence the ultracentrifugation was performed at 15°C in a L2 65-B Beckman preparative ultracentrifuge using a 40.3 rotor. 4 ml of serum was transferred to a 6.5 ml centrifuge tube and overlayered with physiological saline density 1.006. After centrifugation for 16 hours at 40 000 rpm, the top layer, corresponding to VLDL with density <1.006, was collected by tube slicing. The bottom fraction was transferred to a new test tube, the solution density was raised to 1.063 by addition of a NaCl–NaBr solution, and after mixing the sample was centrifuged for 20 hours at 40 000 rpm. The top layer after the second centrifugation contained LDL and the bottom fraction

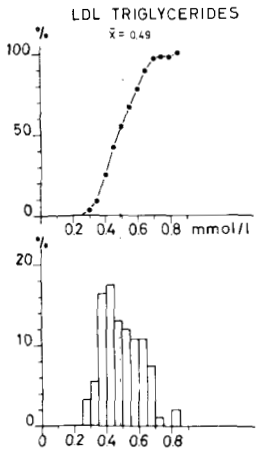

Fig. 6. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of LDL triglycerides in apparently healthy 50-year-old men in Uppsala.

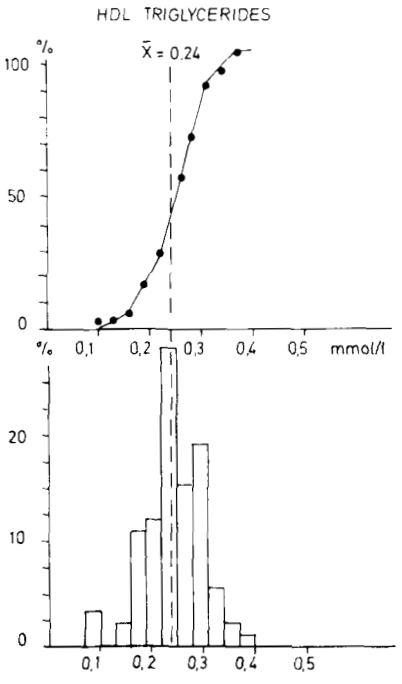

Fig. 8. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of HDL triglycerides in apparently healthy 50-year-old men in Uppsala.

Table I. *Lipid levels in serum LP in healthy 50-year-old men in Uppsala, neg=negative value*

	Triglycerides (mmol/l)			Cholesterol (mg/100 ml)		
	−2 S.D.	Mean	+2 S.D.	−2 S.D.	Mean	+2 S.D.
Whole serum	0.59	*1.80*	3.01	163	*238*	313
Log_{10} whole serum	0.89	*1.71*	3.29		–	
VLDL	neg	*1.03*	2.11	neg	*21*	43
Log_{10} VLDL	0.32	*0.91*	2.59	6	*18*	56
LDL	0.25	*0.49*	0.73	88	*155*	222
HDL	0.12	*0.24*	0.36	24	*48*	73

HDL and remaining serum proteins. Triglyceride and cholesterol concentrations in the LP fractions were determined in isopropanol extracts in a Technicon Auto Analyzer type II (37). A blank was run through the whole preparation procedure and the original serum was analysed at the same time to calculate recovery, the average being 95%. The recovery was 90–110% except in one man where the cholesterol recovery was 88%. The error of the entire ultracentrifugation procedure has shown a within batch coefficient of variation from 4–8% except for HDL triglyceride where it is 15%. The error of the chemical estimation alone is about 3%.

Immediately after centrifugation the top and bottom fractions after the first spin at density 1.006 as well as whole serum were analysed on agarose gel electrophoresis according to Noble (29). A 1% agarose gel containing 0.25% albumin was used. The electrophoresis was run for one hour at a voltage of 16 V/cm in a diemal buffer at pH 8.6, ionic strength 0.05 and stained with Sudan Black after fixation in methanol. All electrophoretic strips were evaluated by two experienced observers independently who had to agree on the interpretation of the pattern if variations from the normal should be recorded.

Grounds for exclusion from the study

The following grounds for exclusion were used to define an apparently healthy population:

1. Clinical or laboratory evidence of disease
2. Not fasting
3. Overweight, weight/height index

$$\frac{\text{weight in kg}}{(\text{height in cm} - 100)} \geq 1.10$$

Ten subjects were excluded because of apparent medical disorders, diabetes mellitus (two), myocardial infarction (one), alcoholism (one), malignant tumour (one) and gastrectomy (two). Other reasons for exclusion were cardiomegalia, elevated sedimentation rate and a low hemoglobin value (8.3 g/100 ml). Three men were not fasting. The LP levels of 26 overweight subjects are reported separately. The remaining 92 apparently healthy men were included in the study.

Statistical methods

Statistical methods according to Snedecor (39) were used to characterize the material. Various estimates of population parameters, frequency and cumulative frequency distribution and regression analysis were computed from standard programs using a MAEL 4000 table computer. Statistical analyses involving serum triglycerides, VLDL cholesterol and triglyceride and LDL triglyceride concentrations were performed also on logarithm transformed values because of the skewed distribution of these variables.

RESULTS

Lipid and LP levels

Figures 1 and 2 show the frequency distribution and the cumulative frequency curves for serum cholesterol and triglycerides. In contrast to cholesterol the triglyceride distribution is skew showing a log-normal distribution. A positive skewness is

Table II. *Lipid levels in serum LP in 50-year-old men in Uppsala*

Percentile values derived from cumulative frequency distribution curves

	Serum		VLDL		LDL		HDL	
Percentile	Chol (mg/100 ml)	TG (mmol/l)	Chol (mg/100 ml)	TG (mmol/l)	Chol (mg/100 ml)	TG (mmol/l)	Chol (mg/100 ml)	TG (mmol/l)
95th	299	3.04	45	2.16	223	0.70	68	0.33
90th	284	2.73	38	1.83	197	0.66	60	0.31
85th	278	2.46	33	1.54	188	0.63	56	0.30
80th	272	2.19	29	1.38	182	0.60	54	0.29

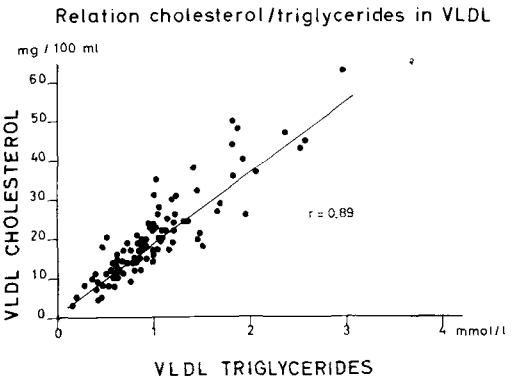

Fig. 9. Relation between cholesterol and triglycerides in VLDL in apparently healthy 50-year-old men in Uppsala.

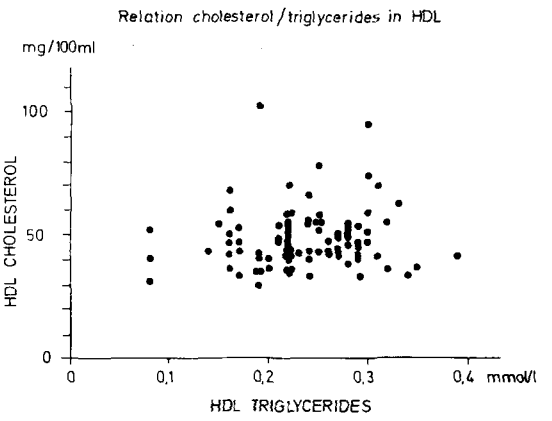

Fig. 11. Relation between cholesterol and triglycerides in HDL in apparently healthy 50-year-old men in Uppsala.

also characteristic for cholesterol (Fig. 3) and triglyceride (Fig. 4) concentrations in VLDL both showing log-normality. The distribution of cholesterol in LDL (Fig. 5) is normal in spite of a tendency for LDL triglycerides to show some positive skewness (Fig. 6). The frequency distribution of HDL cholesterol (Fig. 7) and triglycerides (Fig. 8) are fairly normal although a certain degree of skewness may be found in the cholesterol distribution.

Mean values ±2 standard deviations for cholesterol and triglycerides in serum and in the different LP density classes are given in Table I. Serum triglycerides and VLDL lipid concentrations were also calculated after logarithm transformation. The upper percentile values for cholesterol and triglycerides in serum and in the LP classes were derived from the cumulative frequency curves (Table II).

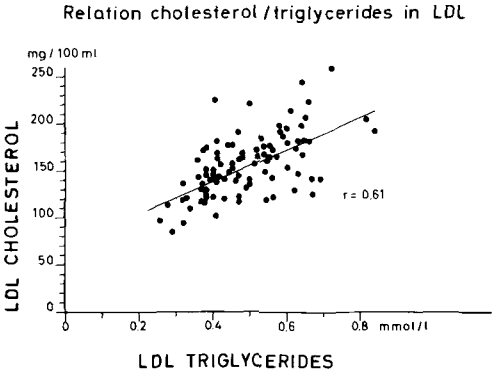

Fig. 10. Relation between cholesterol and triglycerides in LDL in apparently healthy 50-year-old men in Uppsala.

LP composition

The relation between cholesterol and triglycerides in the different LP density classes is shown in Figs. 9–11. There was a significant linear correlation between cholesterol and triglycerides in VLDL (r= 0.89) and in LDL (r=0.61). On the other hand no correlation was found between cholesterol and triglycerides in HDL. The regression line for VLDL but not for LDL contained the origin. Although we are aware of the fact that only VLDL had a linear relation containing the origin between its content of cholesterol and triglycerides, we have for each subject calculated the ratio cholesterol/triglycerides in each LP class. The frequency distributions for the cholesterol/triglyceride ratio

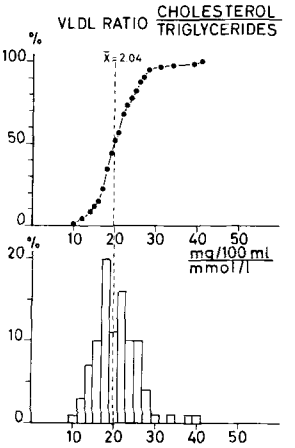

Fig. 12. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of the ratio cholesterol/triglycerides in VLDL.

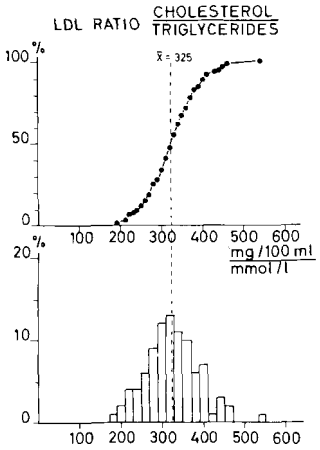

Fig. 13. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of the ratio cholesterol/triglycerides in LDL.

in VLDL, LDL and HDL are shown in Figs. 12–14. At least in VLDL and LDL the ratio is clearly normality distributed. Mean values ±2 S.D. for the cholesterol/triglyceride ratio in the LP density classes and percentile values are presented in Table III.

LP levels in overweight men

The patients with a weight/height index ≥1.10 were excluded from the "healthy" population. When the LP levels in the group of overweight men were determined and compared to the "healthy" men (Table IV) they showed a highly significant increase in triglyceride levels in whole serum as well as in VLDL and a moderately elevated LDL level. The overweight men also showed a substantial increase of serum cholesterol due to a raised VLDL cholesterol. There was no significant elevation of LDL cholesterol.

Table III. *Ratio cholesterol/triglycerides in serum LP in 50-year-old men in Uppsala*

Percentile values derived from cumulative frequency distribution and mean values

	Ratio cholesterol / triglycerides		mg/100 ml mmol/l
Percentile	VLDL	LDL	HDL
95th	29.0	437	375
90th	26.8	400	310
85th	25.2	384	280
80th	24.0	373	260
$\bar{x}$ (S.D.)	20.4 (5.3)	325 (64)	220 (90)
20th	16.6	273	160
15th	15.8	260	150
10th	14.5	243	137
5th	12.5	218	120

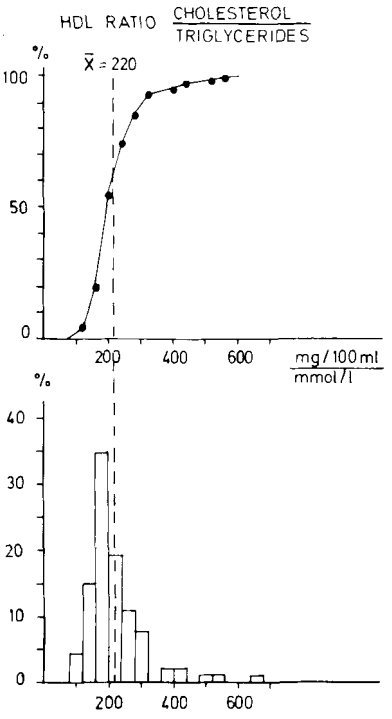

Fig. 14. Frequency distribution (*bottom*) and cumulative frequency distribution curve (*top*) of the ratio cholesterol/triglycerides in HDL.

Although the amount of VLDL was considerably raised the cholesterol/triglyceride ratio was identical with the ratio in healthy men in VLDL and LDL. No significant changes were found in HDL composition.

Limits of "normality" and frequency of HLP

For practical purpose the cut-off points for serum cholesterol and triglycerides, VLDL triglycerides and LDL cholesterol are especially important being the foundation for classification of HLP according to Fredrickson et al. as modified by Beaumont et al. (5). If, for instance, one uses the upper 85th percentile the following rounded off "upper normal" limits are obtained: serum cholesterol 280 mg/100 ml, serum triglycerides 2.5 mmol/l, LDL cholesterol 190 mg/100 ml and VLDL triglycerides 1.5 mmol/l. These cut-off points have been used for classification of the individual LP patterns in this population.

Table IV. *Lipid levels in serum LP in overweight (weight/height ≥1.10) 50-year-old men in Uppsala (n=26)*

	Cholesterol (mg/100 ml)			Triglycerides (mmol/l)			Cholesterol/triglycerides		
	−2 S.D.	Mean	+2 S.D.	−2 S.D.	Mean	+2 S.D.	−2 S.D.	Mean	+2 S.D.
Whole serum	174	*267***	360	0.16	*2.71****	5.26			
VLDL	neg	37***	89	neg	1.81***	3.97	10.6	*19.6*	28.6
LDL	51	*165*	279	0.25	0.55*	0.85	192	*324*	456
HDL	26	*45*	64	0.12	*0.26*	0.30	59	*188*	317

*** (**, *) Significantly increased mean values on the 0.1% (1%, 5%) level when tested against the population of "healthy" 50-year-old men, using *t*-test.

When different percentile limits, derived from the cumulative frequency curves, were chosen as cut-off points for "normality" not only the absolute prevalence of HLP was influenced. Also the relative frequency of different types of HLP varied (Table V). The proportion of combined hyperlipidaemia, type II B according to Beaumont et al. (5), was directly related to the choice of cut-off point as a high limit completely eliminated this pattern from the population.

The estimated frequency of HLP among the obese men was substantially increased compared to the "healthy" men. The relative frequency of the different types of HLP was markedly influenced by the level of cut-off points.

LP composition

A VLDL cholesterol/triglyceride ratio above the 95th percentile of the population (≥29.0 (mmol/l/mg/100 ml)) was seen in two men with normal lipid levels and in two type II A, one of which also presented a "late pre-beta band" (see below) in VLDL and showed some similarities to a type III HLP but with moderately expressed abnormality not diagnostic for this rare disorder. A LDL ratio over the 95th percentile (≥437) was limited to men with a normal LP pattern or an increased beta LP fraction. A LDL ratio below the 5th percentile (≤218) was seen only in subjects with a concomitant increase in VLDL and in some normals also showing a "late pre-beta band" in VLDL. A HDL ratio below the 5th percentile of the population (≤120) was only seen in association with an increased VLDL triglyceride level.

Table V. *Frequency (%) of HLP among apparently healthy and overweight 50-year-old men when different percentile values for LDL cholesterol and VLDL triglycerides were chosen as cut-off points for HLP*

The numbers of subjects in the groups are given in parentheses

Cut-off point (percentile)	HLP type			
	II A	II B	IV	Total
Apparently healthy men (92)				
85th	10.8 (10)	3.3 (3)	12.0 (11)	26.0 (24)
95th	3.3 (3)	0	4.3 (4)	7.6 (7)
Overweight men (26)				
85th	11.5 (3)	15.4 (4)	34.6 (9)	61.5 (16)
95th	11.5 (3)	0	30.8 (8)	42.3 (11)

Agarose electrophoresis

Except for the generally recognized alfa, pre-beta and beta band three specified, "atypical" patterns, previously described (7, 12), could be identified in some of the patients. In a few patients a "double pre-beta band" could be seen in whole serum (Table VI) always corresponding to a "sinking pre-beta band" in the bottom fraction at density 1.006. In most cases the "sinking pre-beta band" could be visualized only when the bottom fractions at 1.006 were studied separately, the band was not prominent in whole serum. A "late pre-beta band", dis-

Table VI. *Prevalence of certain LP patterns on agarose electrophoresis in healthy 50-year-old men in Uppsala*

	Number	%	LP fraction analysed
Double pre-beta	3	3	Whole serum
Sinking pre-beta	16	17	Bottom fraction at d 1.006
Late pre-beta	20	22	Top fraction at d 1.006

tinctly separated from the pre-beta fraction with ordinary migration properties, with a mobility usually being slightly more rapid than that of the beta (LDL) LP, was seen in about 20% of all samples in the top fraction at density 1.006. This band was never recognized in whole serum where it probably was obscured by the beta LP fraction with similar migration properties.

DISCUSSION

LP lipids

The log-normal distribution of triglycerides in serum and triglycerides and cholesterol in VLDL is well known (e.g. 8, 14, 19, 4, 27). When the lipid composition in VLDL was studied a highly positive linear relation existed between cholesterol and triglyceride content. The ratio cholesterol/triglycerides showed a normal distribution. The frequency distribution of particle size in VLDL separated by analytical ultracentrifugation shows a unimodal appearance (15). VLDL is a heterogeneous fraction with respect to both physical characteristics and chemical composition (33). Smaller sized particles contain more protein and less triglyceride. The ratio cholesterol/triglycerides will increase with decreasing particle size (33). The finding of a normal frequency distribution for the cholesterol/triglyceride ratio in this material, with a high correlation between the two lipid components, is in agreement with a concept of one population of particles in normal individuals.

The cholesterol in LDL (density 1.006–1.063) showed a normal distribution. LDL triglycerides on the other hand showed a tendency to skewness. There existed also for LDL a positive linear relation between its content of cholesterol and triglycerides. This relation did not contain the origin and therefore the ratio cholesterol/triglycerides is a somewhat artificial concept which will increase with decreasing lipid content of LDL. It is of course possible that there in fact is a curvilinear relation which contains the origin. The reason why the linear relation did not contain the origin and why the correlation was lower than in VLDL is at least partly due to the fact that we are dealing with an LP fraction (density 1.006–1.063) containing two different particle populations. The bulk of particles is found in the LDL_2 fraction (density 1.019–1.063, Sf 0–12) representing the cholesterol-rich catabolic end product of FLDL breakdown (26). A small amount of particles belong to the LDL_1 fraction (density 1.006–1.019, Sf 12–20), an intermediary particle between VLDL and LDL normally present in low amount in serum. LDL_1 contains relatively more triglycerides than LDL_2 and although quantitatively less than in LDL_2 it may be enough to cause a tendency of skewness of the frequency distribution of triglycerides of the total LDL fraction. The cholesterol content of LDL_1 is small compared with LDL_2 cholesterol.

In HDL the distribution of cholesterol and triglycerides was similar for the two lipids. No correlation was found between cholesterol and triglycerides. A reason for the lack of correlation is the fact that the triglyceride analyses in this density class are both less accurate and less specific than in the other density classes. Another explanation is that HDL also contains two or more subpopulations of particles with different composition (38) without the quantitative dominance of one population of particles as seen in LDL.

Normal serum and LP lipids

The question of how to define limits between "normolipidaemia" and "hyperlipidaemia" is always a source of dispute as the limits are of necessity arbitrarily chosen. For adequate classification of LP patterns every community has to define their own cut-off points as LP levels are different in different populations. In principal any reasonable limit may be set but the origin of the limits must be reported when the prevalence of HLP in a population is described. Sometimes the mean value ±S.D. is used to define the "normal" range but we feel that percentile values derived from cumulative frequency distribution curves are preferable as discussed before (19). "Normal" limits based on percentile values have the advantage of not being influenced by a few extreme values which tend to give a great variance and thus improportionally wide limits when the mean ±2 S.D. is used to set cut-off points for the "normal" range.

The mean value for serum cholesterol in this population of middle aged men was similar to that reported by other investigators in control populations (17, 23, 36, 19, 27) although lower values have been reported (42). In some Scandinavian studies, however, higher cholesterol levels have been recorded (14, 25, 4).

The cholesterol values obtained with the auto-

mated technique used (37) agree well with those obtained with the Sperry–Webb method (40). Triglyceride concentrations determined with the Technicon Auto Analyzer type II according to Rush et al. (37) are for whole serum about 0.2 mmol/l higher than those obtained with the Carlson method (10). The probable reason for this has been discussed in detail earlier (34). Even in regard of methodological variations the mean triglyceride concentration in the population studied was higher than what has been reported in control populations in Great Britain (31, 27), USA (19) and in some Scandinavian studies (25, 4). Comparable triglyceride levels have been recorded in earlier Swedish studies from Stockholm (9, 14) and Uppsala (12) as well as in American investigations of total communities (36, 18). In some of these studies no exclusions were done because of disease or overweight. The apparent regional differences in lipid levels may be due to genetic as well as environmental factors.

Little is known about "normal" lipid concentration in the LP density classes separated by preparative ultracentrifugation. Fredrickson et al. have suggested "normal" limits for cholesterol in the different LP classes (17) and recently Lewis et al. described the cholesterol and triglyceride levels in ultracentrifugally separated density classes in a healthy British population (23). The VLDL triglyceride levels in Uppsala (12) were found to be considerably higher than in London. Also the cholesterol values in VLDL and LDL appeared to be elevated although the age groups were not directly comparable. Similarly the American limits for "normality" for cholesterol in the different density classes were low compared with the results from the present study. The data on LP lipid composition obtained in this study agree well with results from an earlier study of healthy men in Uppsala, obese subjects being eliminated (12).

Weight gain is known to be associated with a tendency to hypertriglyceridaemia (3). In this study the overweight men showed a pronounced elevation of the triglyceride levels in serum mainly due to an increased VLDL fraction. The increase of serum cholesterol was attributed to raised VLDL cholesterol. No significant changes were seen in the cholesterol content of the other LP fractions.

Although considerably increased in obesity the VLDL fraction did not show any change in relative lipid composition. This indicated that obesity was associated with the presence of qualitatively normal LP.

Definition of HLP

The prevalence of HLP in a population depends on the cut-off points as shown in Table V. Less well known is the fact that also the relative frequencies of the different types of HLP are due to the choice of limits. When high limits are chosen combined hyperlipidaemia may be completely eliminated (Table V). Although HLP type IIB is rarely associated with extensively elevated lipid levels it is of great interest because of the high degree of atherogenicity of this disorder (11). Varying limits for hyperlipidaemia may be one of the explanations for the somewhat contradicting reports on LP patterns in patients surviving a myocardial infarction. Patterson & Slack (31) using mean +2 S.D. as cut-off point did not find any overrepresentation of type IIB in a British post-infarction population. Rifkind et al. (35), Goldstein et al. (19) and Aro (4) on the other hand, using lower cut-off points, concluded that there existed an overrepresentation of type IIB in this group of patients compared to a control population.

Analysis of total serum levels of cholesterol and triglycerides is in most cases sufficient for practical clinical purposes. Not infrequently, however, the information gained by this determination gives an incomplete picture. An elevated LDL cholesterol level is sometimes found in spite of a normal serum cholesterol because it is combined with a low cholesterol level in other LP fractions. An elevated serum cholesterol is sometimes due to an increase in HDL which, rather than being deleterious to the patient, may be beneficial (28). A concomitant elevation of serum triglycerides and cholesterol can be due not only to the presence of type IIB but also type IIA, III and IV.

LP lipid composition

In VLDL a ratio cholesterol/triglycerides above the upper 95th percentile was present in six men. None of these had VLDL triglycerides above the 85th percentile. This is in accordance with data reported by Hazzard et al. (21) who showed that a high VLDL ratio may exist in samples with normal pre-beta level without a type III pattern but that a high ratio associated with raised VLDL levels was diagnostical for a type III abnormality. A high LDL ratio ($\geq$437) was in this study seen as-

sociated with normal LP levels and in patients with HLP type II. A low LDL ratio (≤218) on the other hand was seen connected with raised VLDL triglycerides but also in three "healthy" normolipidaemic men all of whom exhibited a "late pre-beta" band in VLDL on agarose electrophoresis. Low HDL ratios were only found in patients with an increased VLDL concentration. The finding of low LDL and HDL ratios associated with increased VLDL levels should be related to the finding of low cholesterol levels in LDL and HDL in patients with this LP disorder.

Electrophoretic LP variants

Three different kinds of "extra bands" were occasionally seen on agarose electrophoresis (Table VI). In three men (3%) a "double pre-beta" band was seen in whole serum. This could in all three patients be shown to correspond to a band migrating with pre-beta mobility in the density class >1.006. This band is identical with the Lp(a) LP described by Berg (6). When the LP fraction with density >1.006 was run separately on agarose electrophoresis this band, designated "sinking pre-beta", was seen in sixteen samples (17%) including the three subjects where the band was visible as a "double pre-beta" band in whole serum. The frequency of a "double pre-beta" (probably corresponding to the Lp(a) LP) band has been reported to be increased (30) or not increased (13) in patients with IHD. In a previous study in Uppsala the frequency of "sinking pre-beta" band was 26% in male controls (12) and 38% in IHD (13) the difference not being significant. However, the Lp(a) antigen can be demonstrated in over 80% of a population by immunological techniques (2) so a proposed association between IHD and Lp(a) may be due to an increased quantity of this LP rather than to the presence of the fraction.

A distinct "late (slow) pre-beta" band, clearly separated from the fraction with normal pre-beta mobility in VLDL, was seen in twenty samples (22%). The slow migration may theoretically be due to either increased particle size causing a retardation of the penetration by a gel filtration effect or to a change in LP composition compared to normal pre-beta LP causing different electrophoretic migration properties. There are indications that the "late pre-beta" band represents an accumulation of intermediary particles (41, 12) similar to the "floating beta" LP seen in type III (32) but that the amount is too small to cause a classical type III pattern. Thus normal males but not females with this "late pre-beta" band have a raised ratio cholesterol/triglycerides in VLDL and increased amount of LDL triglycerides (12). Furthermore when "late pre-beta" is present, both in normo- and hyperlipoproteinemia VLDL has an increased ratio cholesterol/triglycerides and LDL is characterized by raised triglyceride concentration (41, 8).

An accumulation of intermediary particles is characterized by an increased ratio cholesterol/triglycerides in VLDL (21) but also a low ratio cholesterol/triglycerides in LDL (41). In this study one of the two men with normal LP pattern but with a high cholesterol/triglyceride ratio in VLDL showed a "late pre-beta" pattern. All normolipidaemic men with a low ratio in LDL also showed this band.

It has to be emphasized that the diagnosis of atypical electrophoretical patterns is depending on the type and concentration of the supporting medium and the technique used at the separation.

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