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Detection and Characterization of Hyperlipoproteinaemia in Middle-aged Men

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ABSTRACT

Serum lipoprotein (LP) concentrations were determined and LP patterns were classified in 261 middle-aged men, recruited from a health examination survey, with serum lipid values above the 80th percentile of the same population. Individuals with hyperlipoproteinaemia (HLP) and normolipidaemic controls were characterized also regarding family history of cardiovascular disease, socio-economic factors and clinical and laboratory variables. Subjects with HLP type IV–V and IIB were overweight and showed hyperuricaemia and hyperinsulinaemia compared with normolipidaemic controls and subjects with HLP type IIA. The latter showed elevated erythrocyte sedimentation rate. In spite of being overweight, subjects with HLP type III showed normal fasting values of insulin and uric acid in serum and normal early insulin response to intravenous glucose. The glucose tolerance did not differ significantly between the groups. Men with HLP types IV–V had predominantly sedentary occupations, in contrast to those with type IIA. There were significantly more smokers in the groups with HLP type IIB and IV–V than in the control group. Thus, individuals with different types of HLP tend to show different metabolic profiles but also different socio-economic and clinical patterns, suggesting that exogenous factors are of importance in the expression of the LP abnormalities.

INTRODUCTION

In prospective studies, several risk factors for the development of atherosclerotic coronary heart disease (CHD) have been identified. These factors include elevated concentrations of cholesterol (21) and triglycerides (11) in serum. The hyperlipoproteinaemias (HLP) are, according to Fredrickson et al., divided into separate types with different lipoprotein (LP) patterns (16). Etiologically, HLP may be primary, due to genetically determined metabolic defects, or secondary to disorders such as diabetes mellitus and hypothyroidism. However, environmental factors, e.g. dietary habits, stress, physical activity, may also influence the serum lipid levels.

This report concerns a study of the serum LP composition in middle-aged men with hyperlipidaemia recruited from a health examination survey. Subjects with different types of LP pattern were characterized regarding family history of cardiovascular disease, smoking habits, physical activity and socio-economic factors. The association between different types of HLP and some clinical and laboratory variables were also studied.

MATERIALS

A health examination was offered to all men living in the city of Uppsala and born in 1920–24 (19). A total of 2322 men were examined, giving a participation rate of 83.9%. The present study comprised all men analysed between Sept. 1971 and Sept. 1973 with hyperlipidaemia defined as the mean of two values of serum cholesterol and/or triglycerides (TG) above the 80th percentile in this population (291 mg/100 ml and 2.63 mmol, respectively. The hyperlipidaemic subjects were invited to a special lipid clinic for lipoprotein analysis. The following individuals were excluded: 23 men with hypertension, supine diastolic blood pressure (DBP)$\geq$105 mmHg, 3 men with treated diabetes mellitus and one man with untreated hypothyroidism. A total of 270 men were invited to the lipid clinic but 9 of them did not show up.

Ten of the 261 subjects with hyperlipidaemia suffered from medical disorders. Two of them had angina pectoris and one had suffered from a myocardial infarction. One case each of intermittent claudication, operated aortic coarctation, Morbus Crohn, and Morbus Parkinson were included. Three men were treated with drugs: one hyperlipidaemic, one man with corticosteroid treated bronchial asthma and one with epilepsy.

A control group consisting of 100 men, hypertensives excluded (supine DBP$\geq$105 mmHg), with serum lipid values below the 80th percentile at the initial examination were randomly selected: 5 men consecutively examined on the 15th of each month throughout the investigation were chosen. The laboratory and clinical variables as well as socio-economic data of this group were compared with those of the subjects with different types of HLP.

The LP values in the different types of HLP were also

compared with the corresponding values in a randomly selected group of 92 apparently healthy men from the same population study (12).

METHODS

Lipid and lipoprotein analyses

All serum lipid and LP analyses were performed at the Department of Geriatrics, University of Uppsala. Blood samples were drawn after an overnight fast and serum was collected by low speed centrifugation. EDTA was added to a final concentration of 0.05%. Separation of LP density fractions by preparative ultracentrifugation according to Havel et al. (18) was begun on the same day. After centrifugation at 15°C for 16 hours at 40000 rpm using a 40.3 rotor the top fraction corresponding to the very low density lipoprotein fraction (VLDL, $d<1.006$) was collected. From the bottom fraction at $d=1.006$ the low density lipoprotein (LDL, $d=1.006$–1.063) and high density lipoprotein (HDL, $d>1.063$) fractions were isolated either by a second spin at $d=1.063$ in the preparative ultracentrifuge or by precipitation of LDL by a heparin–manganese chloride solution (6). Cholesterol and TG analyses in serum and in the isolated density fractions were performed by semi-automatic techniques in a Technicon Auto-Analyzer type II according to Rush et al. (27). The recovery of the LP analysis, i.e. the sum of cholesterol and TG in VLDL, LDL and HDL, was always within 100 ± 10% of total serum cholesterol and TG, respectively.

Immediately after the ultracentrifugation the top and bottom fractions recovered at $d=1.006$ as well as whole serum were subjected to agarose electrophoresis according to Noble (25). A 1% agarose gel containing 0.25% albumin was used and the chromatogram was stained in Sudan black.

The LP patterns were classified according to Fredrikson et al. using the recommendations by Beaumont et al. (2). Limits for HLP corresponding to the 85th percentile in a random sample of healthy men from the same population study were 190 mg/100 ml and 1.50 mmol/l for LDL cholesterol and VLDL respectively (12). The definition of type III HLP was based on the presence of a slow-moving band in VLDL on agarose gel electrophoresis, with beta or close to beta mobility, in combination with a high ratio cholesterol/TG in VLDL and a high "III-index" (29).

In 6 individuals no complete ultracentrifuge analysis was obtained because of a low recovery of TG in the LP density fractions. However, the information gained from the analyses performed including serum TG and cholesterol concentrations, cholesterol concentrations in the LP classes and the results of the agarose electrophoresis was sufficient for an adequate classification of the LP pattern.

Other laboratory investigations

The analyses of serum uric acid (SUA), erythrocyte sedimentation rate (ESR) and haematocrit were performed with the methods used routinely at the Department of Clinical Chemistry, University Hospital, described elsewhere (19).

The intravenous glucose tolerance test was performed as described before (19). The subjects were asked to be fasting after midnight the day before the examination. No other dietary prescriptions were given.

Serum insulin was determined in duplicate with the Phadebas Insulin Test (Pharmacia AB, Uppsala, Sweden). This method is based upon the radioimmunosorbent technique, described by Wide et al. (30). The early serum insulin response to intravenous glucose was expressed as the mean value of the serum insulin concentrations determined at 4, 6 and 8 minutes after the start of the glucose injection. The late serum insulin response was expressed as the value at 60 minutes. The serum insulin index was defined as the ratio between early insulin response and basal serum insulin concentration (5, 31).

Clinical investigations

The supine BP was measured with a mercury manometer after 10 min rest and read to the nearest 5 mmHg. The DBP was recorded when the sound disappeared entirely (Korotkoff phase 5).

The tables of Lindeberg et al. were used for calculation of relative body weight (22). Subscapular skinfold thickness was measured with a Harpenden caliper.

A 12-lead electrocardiogram was recorded and classified according to the Minnesota Code.

Questionnaire

The family history, as well as the information regarding physical activity, was obtained by a self-administered questionnaire *ad modum* Collen (13). The question and the classification of physical activity used have been presented elsewhere (19). The coding of marital status and social group was based on interview reports. The three conventional social classes were used. The information of smoking habits was also recorded by interview.

Statistical calculations

Conventional methods were used for calculation of mean value and standard deviation (S.D.). Significances of differences between mean values were estimated with Student's t-test (two-tailed test). Logarithm transformed values were used when testing the means of serum insulin concentrations, K-values and ESR because these parameters had a skew distribution (19). For the same reason serum TG concentrations, VLDL and LDL TG and VLDL cholesterol concentrations were also tested after logarithmic transformation (12). The χ^2-test with Yates' correction was used for comparison of frequencies. The accepted level of significance was $p<0.05$.

RESULTS

Lipid and lipoprotein analyses

The mean values for serum cholesterol of the 261 men at the initial screening, at the second examination and at the lipid clinic were 292, 299 and 283 mg/100 ml, respectively. The difference between

Table I. *Classification of lipoprotein pattern in 50-year-old men with hyperlipidaemia*

Lipoprotein pattern	Number of subjects	Frequency (%)
Normal	42	16.1
II A	77	29.5
II B	55	21.1
III	17	6.5
IV[a]	68	26.1
V	2	0.7
Total	261	100.0

[a] Complete ultracentrifuge data missing in 6 subjects.

the first two values was not significant. The analyses at the lipid clinic showed significantly lower values than at the initial ($p<0.05$) as well as at the second examination ($p<0.001$). The corresponding values for serum TG were 3.32, 3.21 and 3.04 mmol/l, respectively. These values did not differ significantly. The mean serum cholesterol and TG concentrations of the 23 men who were excluded because of hypertension did not differ significantly from those of the remaining group of hyperlipidaemics.

The classification of the LP patterns are shown in Table I and the lipid concentrations in the serum LP density fractions of the different types of HLP are shown in Table II. The type II A and II B LDL cholesterol is high by definition. Correspondingly, type II B and IV showed high VLDL TG levels. The VLDL cholesterol concentration in type III was considerably higher than in both types II A and II B ($p<0.001$) and also higher than in type IV ($p<0.01$). The LDL cholesterol was lower in type III than in types II A and II B ($p<0.001$), but on the average higher than in type IV ($p<0.01$).

Other laboratory investigations

The mean of ESR was significantly higher in type II A than in the control group (Fig. 1). The same tendency was seen in type II B ($p=0.06$).

The differences in mean haematocrit values were numerically small. However, the values in all HLP groups were significantly higher than in the control group. The highest value, 44.1±2.5%, was obtained in type II B ($p<0.001$). The haematocrit in type II A was 43.5±2.5% ($p<0.01$) and in type IV–V 43.3±2.4% ($p<0.05$), while the controls had a value of 42.5±2.4%.

The mean SUA concentrations were significantly increased in types II B and IV–V compared with the control group as well as with the other two types of HLP (Fig. 1).

The results of the serum insulin determinations are summarized in Fig. 2. There were no significant differences between the mean *K*-values of the five groups.

No significant differences were observed between the group of 42 men with normal LP pattern (Table I) and the control group with regard to laboratory investigations.

Clinical investigations

The mean systolic blood pressure was 137.1±16.3 mmHg in HLP type II B which was significantly higher ($p<0.05$) than in the control group, 131.5±16.3 mmHg. The mean DBP was significantly higher ($p<0.05$) in types IV–V than in the control group. The values were 85.1±9.3 mmHg and 81.7±10.4 mmHg, respectively. No other significant differences were found. The average pulse rate were the same in all groups.

The weight index was significantly higher in types II B, III and IV–V than in the control group as well as in type II A (Fig. 1). Subjects with HLP types II B and IV–V had an increased skinfold thickness compared with the controls. A history of weight gain of more than 10 kg after the age of 30 was reported by 57% and 44% of the subjects with HLP types IV–V and II B, respectively. This was significantly ($p<0.01$) more frequent than among men with type II A (24%) and in the control group (23%).

Electrocardiogram

The resting ECG was classified according to the Minnesota code. There were no reportable items in 54.7% of the ECGs of the subjects with HLP type II A compared with 72.3% in types IV–V. This difference was significant ($p<0.05$). In the control group, 61.0% of the ECGs were without reportable items. However, this seems a somewhat low frequency since in the total population of more than 2000 men of the same age the corresponding frequency was 69.7%. The frequencies of signs of CHD were too low to allow any conclusions.

Aspects on the family history of cardiovascular deaths

The frequencies of parents dead from myocardial infarctions or cerebrovascular lesion did not differ

Table II. *Lipoprotein lipid concentrations in LP density fractions in 50-year old men with different types of hyperlipoproteinaemia and in a sample of healthy men from the same study*

HLP type	*n*	Serum Chol (mg/100 ml)	Serum TG (mmol/l)	VLDL Chol (mg/100 ml)	VLDL TG (mmol/l)	LDL Chol (mg/100 ml)	LDL TG (mmol/l)
II A	77	305±40	2.00 (2.293±0.087)	23 (1.330±0.189)	0.98 (1.974±0.140)	226±37	0.69 (1.832±0.083)
II B	55	315±25	3.24 (2.505±0.071)	43 (1.615±0.117)	2.12 (2.318±0.097)	218±21	0.72 (1.852±0.095)
III	17	321±67	3.74 (2.532±0.176)	92 (1.879±0.267)	2.42 (2.305±0.242)	173±56	0.80 (1.881±0.151)
IV[a]	68	254±39	4.05 (2.561±0.177)	58 (1.697±0.223)	2.95 (2.406±0.209)	145±30	0.63 (1.780±0.132)
Popul. sample	92	238±38	1.80 (2.233±0.142)	21 (1.252±0.246)	1.03 (1.959±0.227)	155±34	0.49 (1.677±0.109)

Log (mean value×100)±S.D. in parentheses.
[a] Ultracentrifuge data missing in 6 subjects.

between the four groups of HLP. Nor were there any differences when compared to the controls. The frequency of a history of diabetes mellitus among parents of men with different types of HLP was similar, around 6%, and did not differ from that of the controls.

Marital status and social class

When comparing marital status no differences were found between the groups of men with different types of HLP and the control group.

There were significantly fewer men with HLP type II A than in the control group who were clas-

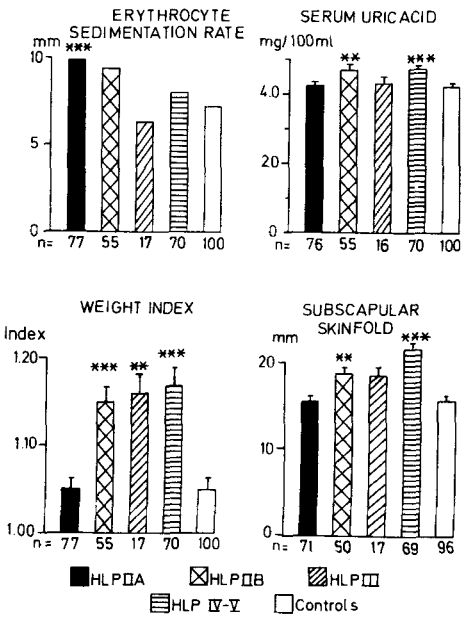

Fig. 1. Erythrocyte sedimentation rate, serum uric acid, weight index and subscapular skinfold thickness in 50-year-old men with different types of hyperlipoproteinaemia (HLP) compared with controls (**$p<0.01$, ***$p<0.001$).

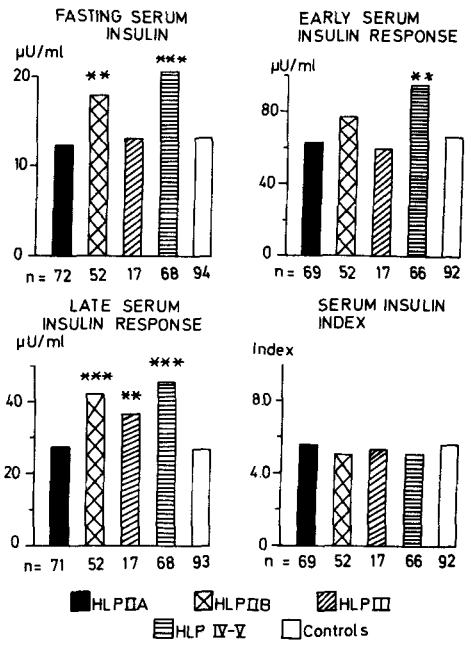

Fig. 2. Serum insulin concentrations in 50-year-old men with different types of hyperlipoproteinaemia (HLP) compared with controls (**$p<0.01$, ***$p<0.001$).

HDL Chol (mg/100 ml)	TG (mmol/l)
47±11	0.25±0.05
42±8	0.28±0.05
41±8	0.34±0.09
37±9	0.31±0.13
48±13	0.24±0.06

sified in social class I (Table III). There was an overrepresentation of men in type IIB classified in social class 2. In type IIA more men belonged to social class 3 than in type IV ($p<0.05$).

Smoking habits

There were 46% smokers in the control group (Table IV). This number corresponded well to the 51% smokers found in the total material of more than 2000 men. The smoking group comprised both cigarette, cigar and pipe smokers. In the groups with types IIB and IV–V there were significantly more smokers than among the controls. This difference was caused by a higher frequency of cigarette smokers (40%) among the men with types IIB and IV–V than in the control group (26%).

Physical activity

The degree of physical activity at work and during leisure reported by the men is shown in Table V. Subjects with HLP type IIA had sedentary work less often than the controls and also in comparison with the subjects with types IIB and IV–V ($p<0.01$). The frequency of subjects with types IV–V who had predominantly sedentary work was significantly increased compared with the controls. Correspondingly, men with type IIA showed the highest frequency of heavy manual work, the difference being significant compared with men with types IV–V ($p<0.01$). In the group with types IV–V, the frequency of men with heavy manual work was significantly lower than in the control group. No significant differences were found when comparing activity during leisure time in the different groups.

DISCUSSION

The purpose of this investigation was to study the association between different types of HLP and case history, clinical and laboratory data. The population was homogeneous with regard to age, sex and geographical factors. The hyperlipidaemic group was unselected with the exception that subjects with hypertension and secondary hyperlipidaemia were excluded.

Serum lipid levels were moderately elevated in these hyperlipidaemics. Only one subject had a serum cholesterol value above 400 mg/100 ml. Most other studies of hyperlipidaemics as well as of LP composition in different types of HLP have been concerned with selected materials of patients with extreme hyperlipidaemia. These studies may not be representative of the moderate HLP found in the general population.

Table III. *Social class classification (%) of 50-year-old men with hyperlipoproteinaemia compared with normolipidaemic controls*

	Lipoprotein pattern			
Social class	IIA ($n=77$)	IIB ($n=55$)	IV–V ($n=70$)	Controls ($n=100$)
1	6.5*	9.1	20.0	19.0
2	35.1	49.1*	42.9	31.0
3	58.4	41.8	37.1	50.0

* $p<0.05$ compared with controls.

Table IV. *Smoking habits (%) among 50-year-old men with hyperlipoproteinaemia compared with normolipidaemic controls*

	Lipoprotein pattern			
Smoking habits	IIA ($n=77$)	IIB ($n=55$)	IV–V ($n=70$)	Controls ($n=100$)
Never smoked	18.2	14.5	17.1	26.0
Ex-smokers	26.0	18.2	20.0	28.0
Smokers	55.8	67.3*	62.9*	46.0

* $p<0.05$ compared with controls.

Table V. *Prevalence of different categories of physical activity at work and during leisure in 50-year-old men with hyperlipoproteinaemia compared with normolipidaemic controls*

Physical activity	IIA ($n=77$)[a]	IIB ($n=55$)[a]	IV–V ($n=70$)[b]	Controls ($n=100$)[c]
At work				
Predominantly sedentary	17.6**	32.7	51.5*	35.7
Predominantly standing and walking	25.7	36.5	30.3	30.6
The same+lifting and handling heavy objects	31.1	11.5	13.6	17.3
Heavy manual work	25.6	19.3	4.5*	16.3
Leisure time				
Inactive	18.9	21.6	15.4	17.5
Some activity	32.4	39.2	44.6	37.1
Regular activity	40.5	39.2	36.9	39.2
Regular hard physical training	8.2	0.0	3.1	6.2

* $p<0.05$, ** $p<0.01$ compared with controls
[a,b,c] Data incomplete in 3, 4 and 2 subjects respectively.

Seventeen subjects (6.5%) of the hyperlipidaemics were classified as type III, corresponding to a prevalence of around 1% in the total population. This seems to be a fairly high prevalence, although comparisons with other studies are uncertain because of dissimilarities in the materials and the criteria used for classification, which in the present study included a qualitative aspect, the requirement of a "floating beta" band in VLDL, a high VLDL cholesterol/TG ratio and a high "III-index" (29).

A high haemoglobin level in men has been reported to be a risk factor for the development of CHD (9, 20, 32). The haematocrit has also been demonstrated to be related to both the later development of myocardial infarction and to death from other causes (28). However, using a multiple logistic model, a high haematocrit reading did not significantly correlate to an increase in events related to CHD (32). A positive correlation between haemoglobin levels and serum lipid levels has been reported and suggested to be dependent on changes in plasma volume (7). Cigarette smokers have higher haematocrit values and haemoglobin levels than non-smokers, presumably because of an impaired respiratory function as well as increased blood levels of carbon monoxide. In our study there was an increased frequency of smokers among men with HLP who were also characterized by higher haematocrit values, compared with the controls.

An elevated ESR in subjects with HLP has earlier been reported in a study of asymptomatic subject with marked hyperlipidaemia (10). An elevated ESR has also been found to be a "risk factor" of CHD (9). The mechanism is unclear. A high LP level has been suggested to influence the ESR through increased binding of lysolecithin (8). Silent vascular disease caused by the hyperlipidaemia has also been discussed as a possible reason for the raised ESR (10).

The SUA concentration was higher in subjects with HLP types IIB and IV–V than in controls. An association between SUA and obesity (14, 23) and hypertriglyceridaemia (15) has been found. Furthermore, in a recent study the SUA level has been demonstrated to be correlated to both the VLDL TG concentration and body weight independently (26).

The average weight index and skinfold thickness in HLP types IIB and IV–V were significantly greater than in the control group and in type IIA, indicating an increased degree of obesity. Supporting this was the finding of a higher frequency of a history of weight gain after the age of 30 in subjects with types IIB and IV–V. Some marginal differences in supine blood pressure could probably be explained by differences in body weight between the HLP types.

It has recently been suggested that there are two forms of obesity (4). One form, acquired in adult

age, is characterized by hypertrophy of fat cells. This type is related to caloric balance and associated with metabolic disturbances. The other form, with onset in childhood, is characterized by an increased number of fat cells. It has been shown that the adipose tissue fat cell size is a more important determinant than the adipose tissue size for the insulin response to glucose tolerance test (3). In this study, significantly more men with HLP types II B and IV–V reported a history of weight gain after the age of 30 than the men in type II A and in the control group. This indicates that there might be more subjects with the hypertrophic type of obesity in types II B and IV–V, which may be an explanation for the hyperinsulinaemia found in these types of HLP.

In contrast to the findings in types II B and IV–V, the HLP type III subjects did not, on average, show elevated fasting serum insulin concentration and early serum insulin response, in spite of a high weight index. Only the late serum insulin response was increased, compared with the controls. The SUA value too was normal in type III. With reservation for the limited number of type III subjects these findings may indicate that the metabolic pattern presumably characteristic of types II B and IV–V is not seen in the type III disorder, which might constitute a pathogenetically different entity.

In contrast to other reports, no significant differences in glucose tolerance were found between the different HLP groups and the control group.

Considering socio-economic data, there seemed to be an association between social class and degree of physical activity at work which might be reflected in the distribution of HLP types.

The socio-economic status may also influence the smoking habits. There were significantly more smokers in HLP types II B and IV–V than in the control group. This is in accordance with the findings in a study in asymptomatic primary hyperlipidaemia (26). The explanation for this association is unclear.

The results of this study indicate that individuals with different types of HLP not only show typical LP pattern but even different socio-economic and clinical patterns, thus supporting the view that exogenous factors are important for the expression of LP pattern abnormalities. The type II A subject is a lean, physically hard working man in social class 3 with elevated ESR but with most of the metabolic parameters normal. The type IV–V subject is an overweight, predominantly sedentary smoker in social class 1 or 2 with elevated SUA and hyperinsulinaemia.

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