Carotid intima-media thickness in young patients with familial hypercholesterolaemia

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Abstract

***Objective*—To assess the extent of early atherosclerotic changes of the carotid arteries in young patients with familial hypercholesterolaemia (FH) detected as increased intima-media thickness (IMT), and to determine the relations between IMT and some clinical and blood variables such as lipid and lipoprotein(a) (Lp(a)) concentration and haemostatic factors.**

***Design*—The IMT of the carotid bifurcation, the proximal 1 cm of the internal carotid artery, and the distal 1 cm of the common carotid artery was determined in all subjects using B mode ultrasonography. Blood lipids, fasting glucose, and several haemostatic variables were also analysed.**

***Subjects*—28 patients with FH (12 males and 16 females aged 11 to 27 years, one homozygote, 27 heterozygotes) and 28 sex and age matched normolipidaemic healthy subjects.**

***Results*—The mean carotid IMT (the average of six measurements of the maximum far wall IMT in the three carotid segments on each side) was significantly greater in patients with FH than in controls (mean (SD) 0·71 (0·15) *v* 0·49 (0·08) mm, $P < 0·001$). In all subjects, the mean IMT was significantly correlated with total cholesterol ($r = 0·59$), low density lipoprotein (LDL) cholesterol ($r = 0·60$), triglycerides ($r = 0·27$), and systolic blood pressure ($r = 0·47$). No correlation was found between the mean IMT and Lp(a), fibrinogen, tissue plasminogen activator, and plasminogen activator inhibitor 1.**

***Conclusions*—The majority of young patients with FH have a greater intima-media thickness of the carotid arteries than healthy subjects. Since the individual susceptibility of patients with FH to increased LDL cholesterol is different, B mode ultrasonography could provide a useful tool to identify those who are more likely to develop premature atherosclerotic disease.**

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Keywords: familial hypercholesterolaemia; carotid intima-media thickness; ultrasonography; fibrinolysis.

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Familial hypercholesterolaemia (FH) is a dominantly inherited disorder caused by mutation of the low density lipoprotein (LDL) receptor gene, resulting in high concentrations of serum LDL cholesterol in early childhood, accelerated atherosclerosis, and premature coronary heart disease.[1] Early atherosclerotic changes of the arterial wall detected as increased intima-media thickness (IMT) can be studied by B mode ultrasonography.[2,3] Because of the association between coronary and carotid artery atherosclerosis and the easy access of the carotid arteries to ultrasound scanning, the examination of extracranial carotid arteries can be used to predict the extent of atherosclerotic involvement of coronary arteries.[4–6] Several investigators have looked for an association between various atherogenic risk factors and intima-media thickening of the carotid arteries by ultrasonographic measurement of IMT. Most studies have been made in middle aged and older subjects,[7–9] while similar attention has not been paid to adolescents and young adults. This would be of particular interest in young patients with FH in whom the development of atherosclerotic lesions is an early event. The objectives of the present study were to compare IMT of the carotid arteries between young patients with FH and well matched healthy controls, and to determine the relations between IMT and some clinical and blood variables, such as lipid and lipoprotein(a) (Lp(a)) concentration and haemostatic factors.

Methods

SUBJECTS

Twenty eight young patients with FH (27 heterozygous and one homozygous) of both sexes (12 males and 16 females) and mean age of 20 years (range 11 to 27 years) were selected among the offspring of patients regularly controlled in a lipid clinic. Heterozygous FH was diagnosed on the basis of raised serum LDL cholesterol and normal triglyceride level according to the criteria of Williams *et al*,[10] based on the age of the subjects and the presence of confirmed FH in close relatives. The diagnosis of the single case of homozygous FH was based on a serum total cholesterol level above 15 mmol/l and the presence of xanthomata before the age of 20.[11] The homozygous patient had had tendon and planar xanthomata before she was treated with LDL apheresis, and her total cholesterol concentration at that time was 19 mmol/l. By the time of the enrolment in the study the xanthomata had regressed and the concentration of total cholesterol had fallen to 12·2 mmol/l. Three

patients were moderate smokers (up to 15 cigarettes per day). Three patients were treated with lovastatin. One patient had arterial hypertension controlled by enalapril. All patients were free of diabetes and signs of cardiovascular disease.

Twenty eight healthy age and sex matched subjects served as the control group. They were normolipidaemic according to the age dependent recommended values of blood lipids.[12 13] Three were moderate smokers. None had hypertension, diabetes, or a family history of premature ischaemic heart disease, defined as one or more first or second degree relatives with myocardial infarction, angina pectoris, or sudden death before the age of 55 (men) or 65 (women). They were taking no drugs.

Each subject was clinically examined before the study. Systolic and diastolic blood pressures were measured twice with a mercury sphygmomanometer after a minimum of 10 minutes rest. The mean was used in the analysis. Body mass index was calculated from the ratio: body weight/(body height)^2 in kg/m^2.

Informed consent was obtained from the subjects above 18 years. In participants aged less than 18 years, the informed consent was signed by their parents. The study was approved by the State ethics committee.

CAROTID ULTRASOUND EXAMINATION

Carotid ultrasound examination was performed using a high resolution B mode Diasonics VST ultrasound system with a 10 MHz linear array transducer. During the examination the subjects lay in the supine position. The B mode scanning protocol involved scanning of the right and left carotid artery. On each side the measurements of the maximum far wall IMT were carried out on three segments, including the proximal 1 cm of the internal carotid artery, the carotid bifurcation, and the distal 1 cm of the common carotid artery. Measurements were made by the examiner using the instrument's electronic calipers. The mean IMT was calculated for each patient as the average of six measurements of the maximum far wall thickness in three carotid segments on each side. All measurements were carried out by the same examiner, blind to the clinical and laboratory data of the subjects participating in the study.

The upper normal value of the mean carotid IMT was defined as 0·6 mm, since 95% of the mean IMT values in controls did not exceed this value. Intima-media thickening was defined as the mean carotid IMT greater than 0·6 mm. Atherosclerotic plaques were defined as focal lesions more than 1 mm thick.

BLOOD SAMPLING

Blood for lipid analysis was sampled on two occasions in a period of four to eight weeks and the mean value of the two measurements was calculated. On the second occasion blood samples for the measurements of glucose, Lp(a), and several haemostatic indices were taken as well. Blood was collected from the antecubital vein between 7 am and 9 am after a 12 hour overnight fast. The second blood sampling was performed after 20 minutes of rest with minimum stasis. Blood for the analysis of glucose, lipids, and Lp(a) was collected without additives. For the analysis of haemostatic indices, the blood was collected in precooled citrated tubes, placed in ice water, and then centrifuged for 30 min at 2000 *g* and 4°C. Plasma was transferred to small plastic vials, frozen in liquid nitrogen, and stored at −70°C until analysed. For the assay of Lp(a), samples of serum were frozen and kept at −20°C until analysed.

LABORATORY METHODS

The concentrations of total serum cholesterol,[14] high density lipoprotein (HDL) cholesterol,[15] triglycerides,[16] and glucose[17] were determined by conventional enzymatic assays. LDL cholesterol was calculated from Friedewald's formula.[18] Lp(a) was measured by Laurell's electroimmunodiffusion method.[19] Fibrinogen in plasma was measured as clottable fibrin.[20] Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1) antigens in plasma were determined by ELISA methods[21 22] using commmercially available kits (Imulyse t-PA and Imulyse PAI-1, respectively, Biopool, Umea, Sweden). Plasma t-PA activity[23] and PAI activity[24] were determined by chromogenic substrate assays (Spectrolyse/fibrin and Spectrolyse/pL, respectively, Biopool, Umea, Sweden). Euglobulin clot lysis time (ECLT) was determined as described elsewhere.[25]

STATISTICAL ANALYSIS

Variables showing a normal distribution were expressed as means and standard deviations and differences between groups were tested with Student's *t* test. Other variables were described by median and range and the differences between groups were analysed by Mann-Whitney U test. For correlation analysis, Pearson's correlation coefficient was calculated for normally distributed variables and Spearman's rank-correlation coefficient for other variables. A possible association between risk factors and mean IMT was analysed by standard multiple regression analysis. The criterion for statistical significance was a P value of less than 0·05. All calculations were performed by the Statistica computer program (Stat Soft Inc, 1992, USA).

Results

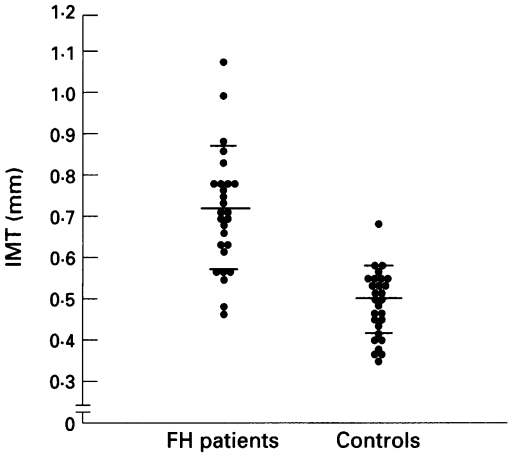
Table 1 shows clinical data and results of blood tests. Patients with FH had significantly higher systolic blood pressure, and higher concentrations of total cholesterol, LDL cholesterol, and triglycerides. Serum Lp(a) levels did not differ markedly between the groups.

The mean carotid IMT was significantly greater in patients with FH than in controls [mean (SD) 0·71 (0·15) *v* 0·49 (0·08) mm, $P < 0·001$] as shown in the figure. In the FH group, 21 patients had increased mean IMT and seven had normal IMT, while in the control group only one subject had an increased

Table 1 Clinical and laboratory characteristics of patients with familial hypercholesterolaemia (FH) and healthy controls. The age is presented as a mean value and range, total cholesterol, LDL cholesterol, HDL cholesterol and glucose are presented as means and standard deviations, other values as medians and ranges

Variable	*FH patients (n = 28)*	*Controls (n = 28)*	*P*
Age (years)	19·9 (11 to 27)	20·4 (11 to 27)	NS
Sex (male/female)	12/16	12/16	NS
Smoking (number)	3	3	NS
Systolic blood pressure (mm Hg)	118 (11)	110 (11)	< 0·01
Diastolic blood pressure (mm Hg)	78 (10)	77 (10)	NS
Body mass (kg)	62·4 (14·4)	64·3 (13·2)	NS
Body height (cm)	168 (11)	172 (11)	NS
Total cholesterol (mmol/l)	8·3 (1·7)	4·1 (0·6)	< 0·001
LDL cholesterol (mmol/l)	6·5 (1·8)	2·3 (0·5)	< 0·001
HDL cholesterol (mmol/l)	1·4 (0·3)	1·5 (0·4)	NS
Triglycerides (mmol/l)	0·9 (0·3 to 2·1)	0·5 (0·3 to 1·0)	< 0·001
Lipoprotein(a) (mg/l)	197 (0 to 1130)	100 (0 to 1348)	NS
Glucose (mmol/l)	4·8 (0·5)	4·8 (0·5)	NS
Fibrinogen (g/l)	2·70 (2·11 to 3·75)	2·87 (2·11 to 3·48)	NS
t-PA antigen (ng/ml)	4·6 (1·9 to 7·6)	4·3 (2·6 to 10·3)	NS
t-PA activity (IU/ml)	1·0 (0·4 to 2·5)	1·0 (0·4 to 3·0)	NS
PAI-1antigen (ng/ml)	9·2 (2·1 to 46·3)	8·8 (2·1 to 35·2)	NS
PAI activity (IU/ml)	5·6 (0·0 to 31·0)	6·8 (0·0 to 24·8)	NS
Euglobulin clot lysis time (min)	202 (75 to 385)	228 (70 to 425)	NS

LDL, low density lipoprotein; HDL, high density lipoprotein; t-PA, tissue plasminogen activator; PAI, plasminogen activator inhibitor.



Graph showing mean carotid intima-media thickness (IMT) in patients with familial hypercholesterolaemia (FH) and in healthy controls. Mean IMT in FH patients was 0·71 (0·15) mm and in healthy controls it was 0·49 (0·08) mm [means (SD)] (P < 0·001).

value of IMT. Nine patients with FH and none of control subjects had atherosclerotic plaques.

Seven patients with FH and normal IMT differed significantly from the other patients only in lower systolic blood pressure values [mean (SD) 111 (9) *v* 120 (11) mm Hg, P < 0·05]. Nine patients with plaques had higher Lp(a) concentrations [median (range) 552 (19 to 1130) *v* 83 (0 to 982) mg/l, P < 0·05] and lower triglyceride concentrations [median (range) 0·6 (0·3 to 1·9) *v* 0·9 (0·4 to 2·1) mmol/l, P < 0·05] than other patients with FH.

In controls, males had significantly greater mean IMT than females [0·53 (0·06) *v* 0·45 (0·07) mm, P < 0·01]. No such sex dependent difference was found in patients with FH [0·72 (0·17) *v* 0·70 (0·14) mm, NS].

All three smokers from the control group had normal IMT. Among three smokers with FH, one had intima-media thickening. In the homozygous patient with FH, several plaques were found and the mean IMT was 0·83 mm.

The correlations of mean IMT with some clinical and laboratory variables are shown in table 2. In all subjects together the mean IMT was positively correlated with systolic blood pressure, total and LDL cholesterol, and triglycerides. In patients with FH the mean IMT was correlated only with age, while systolic blood pressure approached significance. In the control group mean IMT was positively correlated with systolic blood pressure, body height and weight, triglycerides, and t-PA antigen.

The variables that were significant or were

Table 2 Correlations of mean carotid intima-media thickness (IMT) with clinical and laboratory variables in all subjects together and in the group of patients with familial hypercholesterolaemia (FH) and in healthy controls separately on univariate testing

	All subjects		*FH patients*		*Controls*	
Variable	*r*	*P*	*r*	*P*	*r*	*P*
Age	0·22	0·106	0·38	0·048*	0·36	0·064
Systolic blood pressure	0·47	< 0·001*	0·36	0·059	0·40	0·036*
Diastolic blood pressure	0·13	0·352	0·15	0·435	0·11	0·593
Body weight	0·15	0·286	0·19	0·333	0·50	0·007*
Body height	0·07	0·616	0·19	0·339	0·48	0·009*
Body mass index	0·17	0·208	0·14	0·467	0·35	0·072
Total cholesterol	0·59	< 0·001*	−0·05	0·814	0·04	0·849
LDL cholesterol	0·60	< 0·001*	−0·02	0·916	0·19	0·323
HDL cholesterol	−0·16	0·228	0·02	0·911	−0·28	0·154
Triglyceride	0·28	0·038*	−0·21	0·296	0·40	0·036*
Lipoprotein(a)	0·15	0·281	0·09	0·652	0·08	0·692
Glucose	0·03	0·855	0·12	0·531	−0·03	0·886
Fibrinogen	−0·11	0·424	−0·14	0·483	−0·11	0·587
t-PA antigen	0·23	0·086	0·29	0·132	0·45	0·017*
t-PA activity	−0·04	0·773	0·70	0·726	0·07	0·724
PAI-1antigen	0·03	0·829	−0·08	0·703	0·13	0·514
PAI activity	0·01	0·928	0·07	0·719	0·18	0·353
Euglobulin clot lysis time	−0·04	0·781	−0·12	0·528	−0·02	0·302

IMT, intima-media thickness; t-PA, tissue plasminogen activator; PAI, plasminogen activator inhibitor.
*P < 0·05.

Table 3 Multiple linear regression analysis of six variables in predicting mean carotid intima-media thickness (IMT) in all subjects

Variable	*Partial coefficient*	*P*	R^2
Group	0·78	< 0·0001	
Triglyceride	−0·29	0·009	
Age	0·22	0·016	0·61
Systolic blood pressure	0·21	0·052	(P < 0·0001)
t-PA antigen	0·17	0·073	
Sex	0·13	0·196	

t-PA, tissue plasminogen activator.

Table 4 Multiple linear regression analysis of three variables in predicting mean carotid intima-media thickness (IMT) in healthy subjects

Variable	*Partial coefficient*	*P*	R^2
Sex	0·47	0·003	0·47
Age	0·45	0·004	(P < 0·001)
Triglyceride	0·33	0·03	

approaching statistical significance in the univariate analysis were included in the multiple regression analysis. When LDL cholesterol, triglycerides, systolic blood pressure, sex, age, and t-PA antigen were entered into the model, LDL cholesterol ($r = 0·56$, $P < 0·001$) and systolic blood pressure ($r = 0·28$, $P < 0·05$) emerged as independent determinants of the mean IMT. This model explained 44% of the variation of mean IMT ($P < 0·001$). When the group assignment (FH or control group) was entered in the analysis instead of LDL cholesterol, 61% of the variation of the mean IMT ($P < 0·001$) could be explained by the group assignment, triglycerides and age (table 3).

The multiple regression model that explained the greatest portion of the variation of the mean IMT in healthy subjects is shown in table 4. Sex, age, and triglycerides explained 47% of the variation of the mean IMT ($P < 0·001$).

Discussion

The aim of our study was to investigate the intima-media thickness of the carotid arteries in young patients with FH and compare the findings with those of well matched healthy normolipidaemic controls. In 28 healthy controls with a mean age of 20 years the mean IMT was smaller than 0·6 mm in all but one subject. Therefore the 95th centile—that is, 0·6 mm—was taken as the upper limit of normal IMT. We could find no published reference values of IMT for this age group. In a study on Eastern Finnish men aged from 42 to 60 years[7] the average carotid IMT was between 0·53 and 2·25 mm [mean (SD) 1·02 (0·28) mm]. In healthy Japanese subjects without risk factors for atherosclerosis, aged from 24 to 74 years, mean IMT of 0·59 (0·15) mm has been reported by Handa *et al.*[8]

The IMT of the carotid arteries was significantly increased in our subjects, being greater than 0·6 mm in 75%. In addition, atherosclerotic plaques were found in 25% of FH patients, but in none of the controls. In the single study on young FH patients Spengel *et al*[26] reported that 70% of 44 FH patients aged 2 to 29 years (mean 16·2 years) had carotid plaques detectable by duplex scan, while only 12% of the controls were affected. IMT was not measured in that study.

In our study FH patients with normal IMT had lower systolic blood pressure than the FH patients with increased IMT, although all the values were well within the normal range. A tendency to lower values of t-PA antigen and total and LDL cholesterol was also found in FH patients with normal IMT, but these values were not statistically different from the values in other FH patients, perhaps due to the small number of subjects.

Univariate correlation analysis, including both FH and control groups, showed a positive correlation of IMT with total and LDL cholesterol, systolic blood pressure, and triglyceride concentration. Multivariate analysis, including the group assignment instead of the actual values of LDL cholesterol, explained 61% of the variation of the mean IMT by the presence or absence of FH, triglycerides, and age, but almost all of this explained variation was due to the diagnosis of FH.

IMT in healthy subjects was correlated with body height, weight, systolic blood pressure, triglyceride concentration, t-PA antigen, and marginally with age and body mass index. The intima-media complex was thicker in men than in women. In multivariate analysis only male sex, age, and triglyceride concentration were positively related to IMT. These results provided the expected conclusion that IMT values in growing healthy youngsters depended primarily on body size as determined by height, weight, sex, and age. However, the contribution of several risk factors, even within so called normal limits, was also indicated. Among fibrinolytic variables only t-PA antigen, which is believed to indicate endothelial damage,[27] was correlated with carotid IMT.

Univariate analysis in FH patients showed only one variables that was related to IMT, which was age. However, t-PA antigen values in the FH patients with normal IMT were lower than in the other FH patients. The fact that the IMT was almost independent of anthropometric variables can be explained by the major influence of raised cholesterol on the intima-media thickening: its impact may be so predominant in FH patients that it masks the contribution of other factors.

We found no correlation between IMT and Lp(a) concentration. There was also no significant difference in Lp(a) concentration between the FH patients and controls, but FH patients with atherosclerotic plaques had higher Lp(a) levels than the other FH patients. This may indicate that a high plasma Lp(a) concentration is not involved in the development of early changes of the arterial wall preceding atherosclerotic lesions but contributes to a faster progression of atherosclerosis. Correspondingly, in another study in subjects with plaques in the carotid arteries detected by Doppler ultrasonic imaging, higher serum Lp(a) concentrations were found than in subjects with no plaques.[28] A correlation was also

found between serum Lp(a) concentrations and coronary artery stenosis documented by angiography.[29] In the study of Tatò *et al*[30] Lp(a) was found to be the lipoprotein index with the highest discriminative power for the presence of a pathological duplex scan in FH patients older than 30 years.

In conclusion, young patients with FH have a significantly thicker intima-media complex in the carotid arteries than healthy subjects. However, individual response is variable and greater than in healthy individuals. Since the increased IMT is considered to be an early stage of atherosclerosis, the regular follow up of IMT changes in individuals at high risk may be warranted. If the association of the increased carotid IMT with atherosclerosis in other vital locations is confirmed in prospective studies, the ultrasound examination of carotid arteries may serve as a non-invasive test to identify those who are more likely to develop premature ischaemic coronary, cerebrovascular, or limb disease, and to monitor the effect of medical intervention aimed at the regression of atherosclerosis during its early stages.

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