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Mild-to-Moderate Hypertriglyceridemia in Young Men Is Associated With Endothelial Dysfunction and Increased Plasma Concentrations of Asymmetric Dimethylarginine

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OBJECTIVES The aim of this study was to investigate endothelial function and common carotid intima-media thickness (IMT) in healthy young men with mild-to-moderate hypertriglyceridemia. Plasma asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, was measured to further elucidate the mechanisms involved.

BACKGROUND Hypertriglyceridemia is a risk factor for coronary heart disease although the mechanisms behind the increased risk remain to be defined. Acute elevation of plasma triglycerides induced by an intravenous fat load is associated with impaired endothelial function. The results of studies examining acute effects induced by a high-fat meal or effects of chronic hypertriglyceridemia on endothelial function are more inconsistent.

METHODS Flow-mediated vasodilation and nitroglycerin-induced vasodilation of the brachial artery and common carotid IMT were measured noninvasively by ultrasound technique in 15 hypertriglyceridemic (HTG) subjects and 15 matched controls, mean age 34 years. Plasma concentrations of ADMA were measured by high-performance liquid chromatography.

RESULTS Flow-mediated vasodilation was decreased in the HTG group ($p < 0.0001$), whereas nitroglycerin-induced vasodilation and carotid IMT did not differ significantly. Asymmetric dimethylarginine concentrations were higher in the HTG group ($p < 0.05$).

CONCLUSIONS Hypertriglyceridemia in young men is associated with endothelial dysfunction and increased plasma concentration of ADMA but not with increased IMT of the common carotid artery. The corollary is that chronic hypertriglyceridemia results in endothelial dysfunction, possibly due to increased ADMA concentration, and that endothelial dysfunction might precede increased IMT among the early manifestations of atherosclerosis. (J Am Coll Cardiol 2001; 38:111–6) © 2001 by the American College of Cardiology

Hypertriglyceridemia is a risk factor for coronary heart disease (CHD) although the biological mechanisms behind the increased risk remain to be properly defined (1). For instance, it is unclear whether atherogenic or thrombogenic factors are involved or a combination thereof. Endothelial dysfunction and increased common carotid intima-media thickness (IMT) are both early features of atherosclerosis (2–4). Common risk factors for CHD, such as age, smoking, hypertension, hypercholesterolemia and diabetes mellitus are associated with impaired endothelium-dependent vasodilation (5–9), which supports a pathogenic role of endothelial dysfunction in atherosclerosis. Patients with manifest CHD have impaired endothelium-dependent vasodilation in brachial as well as in coronary arteries, with a close correlation between the two sites, indicating a systemic nature of endothelial dysfunction (10,11). Several risk factors for CHD are also associated with increased carotid IMT (12,13), and it has been shown that IMT measures correlate with endothelial function (14). Carotid IMT measured by high-resolution ultrasound imaging technique is increasingly used as a marker of early atherosclerosis, and it is associated with the degree of coronary artery disease (15,16).

In a previous study of healthy young men, we showed that endothelium-dependent flow-mediated dilation (FMD), measured by ultrasound of the brachial artery, decreased after experimental triglyceridemia produced by infusion of a triglyceride-rich fat emulsion (17), whereas data regarding FMD after a high-fat meal are inconclusive (18,19). Data regarding endothelial function in chronic hypertriglyceridemia are also inconsistent. Chowienczyk et al. (20) showed in 1997 that patients with severe hypertriglyceridemia, due to low lipoprotein lipase (LPL) activity, have normal pharma-

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Abbreviations and Acronyms

ADMA = asymmetric dimethylarginine
BMI = body mass index
CHD = coronary heart disease
FFA = free fatty acids
FMD = flow-mediated vasodilation
HDL = high-density lipoprotein
HTG = hypertriglyceridemic
IMT = intima-media thickness
LDL = low-density lipoprotein
LPL = lipoprotein lipase
NTG = nitroglycerin
VLDL = very low-density lipoprotein

cologically stimulated endothelium-dependent vasodilation, as assessed by forearm venous occlusion plethysmography (20). More recently, two studies using the same technique have shown that moderate (21) and severe hypertriglyceridemia, but with normal LPL activity (22), are associated with impaired endothelium-dependent vasodilation. Two studies have hitherto been performed to noninvasively investigate effects of chronic hypertriglyceridemia on endothelial function using FMD of the brachial artery with conflicting results (23,24). Whether hypertriglyceridemia is accompanied by increased IMT, on the other hand, has not been extensively studied, and no such studies have been performed in young men with mild-to-moderate hypertriglyceridemia.

This study was conducted to investigate the relations between chronic mild-to-moderate hypertriglyceridemia in young men and two markers of early atherosclerosis. To the best of our knowledge, this is the first study that has simultaneously investigated FMD of the brachial artery and common carotid IMT in relation to a risk factor for atherosclerosis. Since the young hypertriglyceridemic (HTG) men showed impaired FMD, the plasma levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, were also measured to further elucidate the mechanisms behind endothelial dysfunction in hypertriglyceridemia.

METHODS

Subjects. Healthy men under the age of 40 years with a fasting plasma triglyceride concentration between 3 mmol/l and 10 mmol/l and a fasting plasma cholesterol concentration below 6.5 mmol/l were invited to participate in the study. They were identified in a screening program directed towards employees of private companies in the Stockholm metropolitan area. Healthy male controls matched for age and body mass index (BMI) who had fasting plasma concentrations of triglyceride below 1.6 mmol/l and cholesterol below 6.5 mmol/l were recruited among hospital staff and volunteering friends. Two of the volunteering controls were found to be HTG and were included in the HTG group. All participants had to be normoglycemic (fasting plasma glucose <6.2 mmol/l) and normotensive with a systolic blood pressure below 140 mm Hg and a diastolic blood pressure below 90 mm Hg. Further exclusion criteria were smoking, excessive alcohol consumption, any current medication and a family history of premature CHD.

Study protocol. Fifteen HTG and 15 control subjects were finally included in the study. Because of the day-to-day variation in plasma triglycerides and FMD, all subjects were examined on two separate occasions within one week. The participants arrived in the laboratory the morning after an overnight fast. They were placed in a supine position in a quiet darkened room with a temperature of 22°C to 23°C before venous blood was collected. Ultrasound imaging of the left and right common carotid arteries was followed by ultrasound measurement of the left brachial artery, performed according to protocols described below. All ultrasound scans and images were recorded on videotapes.

The Ethics Committee of the Karolinska Hospital approved the study, and all subjects gave their informed consent to participate.

Blood sampling and lipid, free fatty acid (FFA), insulin and ADMA determinations. Venous blood was drawn into vacutainer tubes containing 1.4 mg Na_2EDTA/ml and instantly put into ice water. Plasma was recovered after low-speed centrifugation (1.750 *g*, 20 min, +4°C). Samples for analyses of glucose, insulin, FFA and ADMA were immediately frozen at −20°C and then transferred to −70°C. On thawed samples, determinations were made of plasma glucose concentrations by chemical methods (Vitros, Johnson & Johnson AB, Sollentuna, Sweden) and of plasma insulin and FFA concentrations by enzymatic methods (DAKO insulin, DAKO Diagnostics Ltd, Cambridgeshire, United Kingdom and NEFA C, Wako Chemicals GmBH, Neuss, Germany). Frozen plasma samples were transported on dry ice to Stanford University, Stanford, California, where plasma concentrations of ADMA were measured on thawed samples by high-performance liquid chromatography, as described (25). The recovery rate for ADMA was 84%, and the coefficient of variation was 3.5%. The detection limit of the assay was 0.1 μmol/l.

Concentrations of cholesterol and triglycerides in very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) fractions were determined in fresh plasma by a combination of preparative ultracentrifugation and precipitation of apo B-containing lipoproteins followed by lipid analyses (26). Cholesterol and triglyceride concentrations were determined by enzymatic methods (Triglycerides/GB, Boehringer Mannheim Corp, Indiana, and Unimate 5 Chol, Triolab, Mölndal, Sweden).

Endothelial function. Brachial artery flow velocity, endothelium-dependent FMD and endothelium-independent nitroglycerin (NTG)-induced dilation were examined according to the method described by Celermajer et al. (5). The measurements were made noninvasively using a high-resolution ultrasound scanner (Acuson 128 XP/10 c, Mountain View, California) with a 7-MHz linear array

transducer. Baseline measurements of blood flow and inner diameter of the artery were performed at rest. Reactive hyperemia was obtained by distal forearm artery occlusion with a 12.5-cm blood pressure cuff at the wrist inflated to 300 mm Hg for 4.5 min. Blood velocity was measured immediately after cuff release, and the inner diameter of the artery was measured 50 to 60 s after deflation. New inner diameter measurement was performed after a 10-min rest followed by administration of 0.4-mg sublingual NTG. After 4 min, blood velocity and inner diameter measurements were repeated. All analyses of the inner diameters of the brachial artery were performed by one investigator who was blinded to the identity of the subject, date and sequence of the ultrasound scan. Four sequential diastolic frames taken coincidentally with the R wave on the electrocardiogram were analyzed at rest and after each stimulation, and the average diameter of the four frames was calculated. Blood flow was calculated from Doppler velocity, vessel diameter and heart rate. The increase in blood flow after reactive hyperemia is presented as a percentage of basal flow values. In the control subjects, the variation in differences between FMD measurements performed within one week was 2.3 ± 2.5%. The within-individual variations between two determinations of FMD performed during the same day and between three determinations made on separate days in our laboratory are 0.88 ± 0.82% and 3.3 ± 2.7%, respectively, as previously reported (17).

Common carotid IMT. The IMT of both common carotid arteries was measured using the same ultrasound scanner and a similar transducer as for endothelial function measurements. The common carotid arteries were scanned to the level of the bulb, and the far wall thickness was measured over a length of 1 cm just proximal to the carotid bulb. The IMT was defined as the distance between the leading edge of the luminal echo to the leading edge of the media/adventitia echo (26). A frozen longitudinal image, synchronized to the top of the R-wave on the electrocardiogram, was recorded on videotape and later transferred to an automated computerized analysis system (Dept. of Signal and Systems, Chalmers University of Technology, Göteborg, Sweden). It has previously been shown that this system reduces the variability of the ultrasound measurements of IMT (27–29). The echo structures of the ultrasound image were automatically detected and IMT and vessel diameter measured by the computer. One experienced reader who was unaware of the case control status of the subjects performed all measurements. The coefficient of variation of duplicate IMT measurements in control subjects performed within one week were 3.2% and 3.3% for the right and left common carotid artery, respectively. The IMT is presented as the mean value of the left and right common carotid artery IMT.

Statistical methods. Group distributions are expressed as mean ± SD of two determinations. The coefficients of variation were calculated according to standard methods. Statistical testing of differences in continuous variables between groups were made by Student unpaired *t* test for normally distributed variables and by Mann-Whitney *U* test for triglycerides, VLDL triglycerides, HDL cholesterol, insulin and ADMA. A p value <0.05 was considered significant.

Table 1. Clinical and Biochemical Characteristics of the Study Groups

	HTG Subjects (n = 15)	Control Subjects (n = 15)	p Values
Age, yrs	34.0 ± 5.0	34.3 ± 4.5	NS
BMI, kg/m^2	27.5 ± 2.2	27.4 ± 2.0	NS
Blood pressure, mm Hg			
Systolic	124 ± 12.6	123 ± 8.3	NS
Diastolic	79 ± 6.0	80 ± 2.5	NS
Triglycerides, mmol/l	4.03 ± 2.82	1.17 ± 0.26	< 0.0001
VLDL triglycerides, mmol/l	3.54 ± 2.90	0.83 ± 0.29	< 0.0001
LDL cholesterol, mmol/l	2.82 ± 0.46	2.98 ± 0.49	NS
HDL cholesterol, mmol/l	0.82 ± 0.18	1.18 ± 0.21	< 0.0001
Glucose, mmol/l	6.08 ± 0.26	5.91 ± 0.39	NS
Insulin, mmol/l	82.8 ± 105.4	38.8 ± 17.6	< 0.05
FFA, μmol/l	290 ± 94	307 ± 136	NS
ADMA, μmol/l	1.13 ± 0.37	0.92 ± 0.28	< 0.05

Values are expressed as means ± SD calculated from the mean of the two examinations.

ADMA = asymmetric dimethylarginine; BMI = body mass index; FFA = free fatty acids; HDL = high-density lipoprotein; HTG = hypertriglyceridemic; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.

RESULTS

Subjects and biochemical data. The clinical and biochemical characteristics of the studied subjects are presented in Table 1. Hypertriglyceridemic and normotriglyceridemic control subjects were well matched for age and BMI. There were no differences in systolic and diastolic blood pressures, glucose, FFA or LDL cholesterol concentrations. Subjects with hypertriglyceridemia had significantly higher VLDL triglyceride and insulin concentrations and lower HDL cholesterol concentration than controls. Hypertriglyceridemic subjects had higher plasma ADMA concentration than normotriglyceridemic control subjects (Table 1).

Brachial artery determinations. The baseline diameters of the brachial artery were similar in HTG and control subjects (4.12 mm ± 0.40 mm vs. 4.17 mm ± 0.44 mm, NS), and there was a similar increase of blood flow after reactive hyperemia in both groups (500 ± 93% vs. 570 ± 130%, NS). Flow-mediated dilation was decreased in HTG subjects compared with controls (0.6 ± 0.8% vs. 4.5 ± 2.0%, $p < 0.0001$) (Fig. 1). In contrast, there was no difference in NTG-induced vasodilation (16.4 ± 2.6% vs. 17.5 ± 6.2%, NS).

Common carotid measurements. The diameters of the common carotid arteries were similar in the two groups. In HTG subjects, the diameter was 6.29 mm ± 0.58 mm on the right side and 6.15 mm ± 0.47 mm on the left side. The corresponding diameters in the control subjects were 6.34 mm ± 0.38 mm and 6.19 mm ± 0.39 mm, respectively. The mean IMT of the far wall of the right and left common carotid arteries was 0.55 mm ± 0.05 mm in HTG

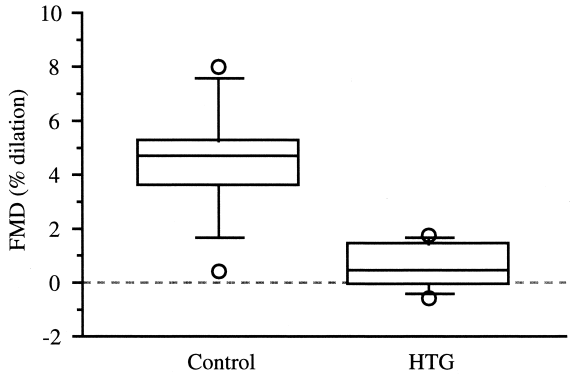

Figure 1. Box plots of relative change in endothelium-dependent flow-mediated vasodilation (FMD) after reactive hyperemia in hypertriglyceridemic (HTG) (n = 15) and control (n = 15) subjects (0.6 ± 0.83% vs. 4.5 ± 2.0%, $p < 0.0001$). The box plot displays 25th, 50th (median) and 75th percentiles in the box and 5th and 95th percentiles as **horizontal lines outside the box**. All values above or below these percentiles are plotted as **points**.

subjects compared with 0.53 mm ± 0.06 mm in control subjects (NS, Fig. 2). There were, likewise, no differences between the two groups when the IMTs of the left and right sides were analyzed separately.

DISCUSSION

The results of this study show that chronic mild-to-moderate hypertriglyceridemia in young men is associated with impaired endothelium-dependent vasodilation measured as FMD of the brachial artery but not with increased IMT of the common carotid artery. This suggests that hypertriglyceridemia is associated with premature atherosclerosis and that FMD of the brachial artery might be a more sensitive measurement of early atherosclerosis than common carotid IMT.

Hypertriglyceridemia and endothelial function. The results of earlier studies of endothelial function in chronic hypertriglyceridemia are inconsistent. In the study by Chowienczyk et al. (20), patients with severe hypertriglyceridemia and low LPL activity had normal pharmacologically stimulated endothelium-dependent vasodilation examined by forearm venous occlusion plethysmography. In contrast, two more recent studies using the same technique have demonstrated an impaired endothelium-dependent vasodilation in subjects with either moderate (21) or severe hypertriglyceridemia and normal LPL activity (22). Two studies have been published so far using FMD measured by ultrasound of the brachial artery for assessment of endothelial function. Lupattelli et al. (24) showed that chronic hypertriglyceridemia was associated with impaired endothelium-dependent vasodilation, whereas Schnell et al. (23) failed to show a difference between cases and controls. The reason for the discrepant results of the previous studies in chronic hypertriglyceridemia is not easily discernible. One possible explanation could be the different techniques used, including the mode of stimulation.

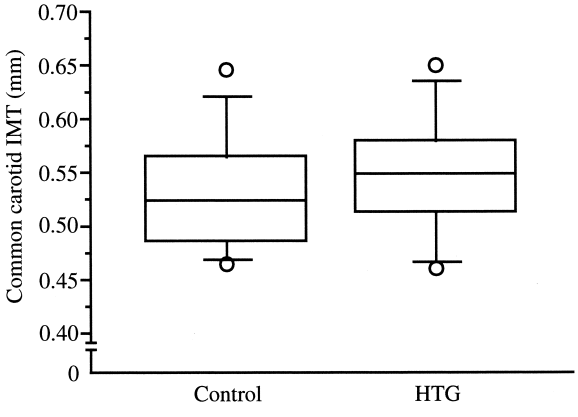

Figure 2. Box plots of common carotid intima-media thickness (IMT) in hypertriglyceridemic (HTG) (n = 15) and control (n = 15) subjects (0.55 ± 0.05 mm vs. 0.53 ± 0.06 mm, NS). The box plot displays 25th, 50th (median) and 75th percentiles in the box and 5th and 95th percentiles as **horizontal lines outside the box**. All values above or below these percentiles are plotted as **points**.

Two important design features differed between this study and that of Schnell and coworkers (23). In the study by Schnell, the two hyperlipidemic groups and the control group contained a fairly large proportion of premenopausal women whose positions in the menstruation cycle at the time of the study were unknown. Secondly, the hyperlipidemic groups were older than the controls. Surprisingly, despite the difference in age, there were no differences in endothelium-dependent vasodilation, neither between the HTG subjects and the control group nor between the hypercholesterolemic individual and the control group. Of note is that previous studies have consistently shown that hypercholesterolemia is associated with endothelial dysfunction (5–7,30).

HDL. There was an expected difference in HDL cholesterol concentration between HTG subjects and controls since increased VLDL triglycerides are strongly associated with low HDL cholesterol (31). It, therefore, cannot be concluded with certainty that the endothelial dysfunction seen in this study is due to hypertriglyceridemia. Previous studies have demonstrated that the HDL cholesterol concentration is related to endothelial function (32,33). However, in the study by Toikka et al. (33), which showed that a decreased HDL cholesterol concentration is associated with endothelial dysfunction, the two groups were neither matched for plasma triglyceride nor for LDL cholesterol concentrations, which differed significantly. To properly assess the impact of HDL on endothelial function in vivo, subjects with different HDL cholesterol concentrations, but matched for plasma triglyceride and LDL cholesterol concentrations, need to be examined.

In this study, correlations were found between both VLDL triglyceride and HDL cholesterol concentrations on the one hand and FMD of the brachial artery on the other (data not shown). Due to the selection criterion for participation in the present case-control study, presence or absence of hypertriglyceridemia, no further conclusions can be drawn from multivariate analysis.

Common carotid IMT. In our study we also examined the common carotid IMT to further assess early stages of

atherosclerosis. Several observations justify the use of this surrogate measurement. Increased IMT is associated with established risk factors for CHD (12,13) and correlates with the severity of coronary artery disease (15,16). There is a paucity of studies of IMT in hypertriglyceridemia. Karpe et al. (34) demonstrated that middle-aged men with hypertriglyceridemia have an increased IMT compared with healthy normolipidemic controls, and studies of postprandial triglyceridemia have shown correlation between the response to a high-fat meal and IMT, independently of other risk factors for CHD (35,36). Furthermore, Grønholdt et al. (37) found that an increased lipid content in carotid plaques, as demonstrated by ultrasound technique, is associated with increased fasting and postprandial plasma triglyceride concentrations. Since hypertriglyceridemia was associated with endothelial dysfunction, which is a marker of early atherosclerosis and possibly also mechanistically implicated in atherogenesis, we suggest that hypertriglyceridemia is a proatherogenic state. No difference in IMT was seen between cases and controls, and it can, therefore, be speculated that endothelial dysfunction might be an earlier feature of atherosclerosis than increased IMT.

ADMA. The HTG subjects had higher plasma concentrations of the endogenous nitric oxide synthase inhibitor ADMA than the control subjects. Increased concentrations of ADMA have been shown to be associated with decreased nitric oxide production and with decreased endothelium-dependent FMD in hypercholesterolemic patients (25). The plasma concentration of ADMA is also increased in patients with other risk factors for CHD (38) and in peripheral arterial disease (39), and it is associated with carotid IMT (40). Furthermore, in patients with type 2 diabetes mellitus, it has recently been shown that ADMA levels increase in response to a high-fat meal, accompanied by impaired FMD (41). The biological mechanisms behind the increased ADMA concentrations are not yet fully understood, but reduced degradation of ADMA may be involved in its accumulation in hyperlipidemia (38). Asymmetric dimethylarginine plays an important role in the regulation of nitric oxide bioavailability and has been suggested to be a novel marker of atherosclerosis.

Conclusions. Hypertriglyceridemia in young men is associated with endothelial dysfunction and increased plasma concentration of ADMA but not with increased IMT of the common carotid artery. We suggest that chronic hypertriglyceridemia results in endothelial dysfunction, possibly due to increased ADMA concentrations and that endothelial dysfunction might precede increased IMT among the early manifestations of atherosclerosis.

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