

Plasma Triglycerides and Type III Hyperlipidemia Are Independently Associated With Premature Familial Coronary Artery Disease

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OBJECTIVES	This study was designed to explore contributions of plasma total triglycerides (TGs) and type III hyperlipidemia to the risk of premature familial coronary artery disease (CAD).
BACKGROUND	Although plasma TGs are recognized as a risk factor for CAD, the independence of this association from related risk factors remains controversial. Also, the degree of CAD risk conferred by excess remnants of TG-rich lipoproteins in type III hyperlipidemia remains unclear.
METHODS	We analyzed lipids by ultracentrifugation in a series of 653 cases with premature familial CAD (myocardial infarction or revascularization by age 55 years in men or age 65 years in women, with similar onset in at least one other first-degree relative) and in 1,029 control subjects. The relationship of CAD risk to various strata of plasma TGs, high-density lipoprotein (HDL) cholesterol, and type III hyperlipidemia, and interactions among these variables were examined by multiple logistic regression, adjusting for other CAD risk factors.
RESULTS	The odds ratio for CAD with elevated plasma TG rose progressively to 11.4 in those with TGs 500 to 799 mg/dl (95% confidence interval 3.4 to 38.0, $p < 0.0001$) compared with <100 mg/dl, even after correction for HDL cholesterol, other elements of the metabolic syndrome, and other CAD risk factors. Risk of CAD associated with type III hyperlipidemia (found in 3.4% of cases) was also markedly increased independent of other risk factors (odds ratios of 5 to 10 depending on the model, all with $p < 0.002$).
CONCLUSIONS	The association between the plasma TG level and premature familial CAD is strong, graded, and independent. Risk of CAD is also strikingly elevated with type III hyperlipidemia. (J Am Coll Cardiol 2005;45:1003–12) © 2005 by the American College of Cardiology Foundation

Elevated plasma triglyceride (TG) concentration is becoming increasingly established as an independent risk factor for premature coronary artery disease (CAD) (1–3). The excess risk is particularly apparent in women (4–7) and in European studies (8–10). The high prevalence of familial TG elevations among premature CAD patients further illustrates the importance of hypertriglyceridemia as a CAD risk factor (11,12). Despite this consensus, uncertainty persists regarding the strength and independence of plasma TGs as a CAD risk factor.

Several lines of evidence suggest that the association of plasma TGs with CAD is complex. In the Prospective Cardiovascular Munster (PROCAM) study, CAD risk increased proportionately with TGs up to 800 mg/dl but began to diminish in persons with levels above 800 mg/dl (10). In addition, the risk associated with TGs ≥ 200 mg/dl was dependent on concomitant low high-density lipoprotein (HDL)-cholesterol or elevated low-density lipoprotein (LDL)-cholesterol/HDL ratio in both PROCAM (13) and

in the Helsinki Heart Study (14). Chylomicronemia may confer low CAD risk despite very high TGs (15,16). In contrast, type III hyperlipidemia, which results from impaired removal of TG-rich lipoprotein remnants, may associate with extreme CAD risk despite relatively modest TG elevations (17,18). Surprisingly, however, published population-based estimates of CAD risk associated with type III hyperlipidemia are entirely lacking. Also, the moderate elevations in TGs seen with metabolic syndrome seem to be associated with moderate CAD risk (19–21).

Uncertainties regarding the independence and strength of plasma TGs as a CAD risk factor in published studies are likely due both to lack of attention to the nature of TG elevations and small study size resulting in inadequate statistical power. We studied a large series of patients with premature familial CAD and population-based controls. We calculated CAD risk across the spectrum of plasma TG levels and tested for interactions with other elements of the metabolic syndrome. Also, for the first time we report a population-based estimate of CAD risk associated with type III hyperlipidemia determined by measurement of plasma lipids by ultracentrifugation in all cases and controls.

METHODS

Study participants. Premature, familial CAD cases consisted of 499 men and 154 women who had survived a myocardial infarction, percutaneous transluminal angio-

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Abbreviations and Acronyms

BMI	=	body mass index
CABG	=	coronary artery bypass grafting
CAD	=	coronary artery disease
CI	=	confidence interval
HDL	=	high-density lipoprotein
LDL	=	low-density lipoprotein
TG	=	triglyceride

plasty, or coronary artery bypass grafting (CABG) by age 55 years for men or age 65 years for women. To minimize artifactual effects of the acute coronary syndrome on lipid levels, patients were seen at least six months after their acute event. Each of the CAD cases was from a family in which at least one additional first-degree relative (parent, sibling, or child) had early CAD by this definition. The CAD cases came from 531 families with the following distribution of screened relatives: 1 family had 6 affected relatives, 1 family had 5, 1 family had 4, 7 families had 3, 96 had 2, and the remaining 425 families had only 1 affected relative screened. These cases were ascertained from two sources: 78% were from discharge records of Intermountain Health Care (which had between 50% and 62% of the health care market share in the state of Utah during the years of recruitment) (22); the remainder were ascertained through the Family Health Tree program (defined in later text). Recruitment of our CAD cases occurred throughout the 1990s, with the majority recruited in the middle of the decade. Initial manifestations of CAD in cases were myocardial infarction in 74.7%, CABG in 13.8%, and percutaneous transluminal angioplasty in 11.5%.

Controls included 488 men and 541 women consisting of two groups. The first group (527 controls) consisted of a random sampling of family members participating in our Family Health Tree Program (23,24). Briefly, high school students in health classes throughout Utah collected health information on family members with the help of their parents. The data were computerized and feedback provided to students and their families regarding familial disease tendencies. Prior samples identified from Family Health Trees have been studied and found to be representative of the Utah population (25). Upon close examination, these controls tended to live in suburban areas. The second group (502 controls) was spouse pairs participating in a study of psychological factors related to CAD. This group was recruited by advertising in newspapers, at conventions, and from the University of Utah community. As acute hemodynamic responses to various stressors were tested as part of that study, persons taking certain vasoactive drugs (beta-blockers, calcium channel blockers, and other anti-anginal medication) were excluded. These controls tended to live in urban Salt Lake City. Control subjects could not have clinically diagnosed CAD themselves, but no effort was made to exclude controls based on family history of CAD.

Comparability of the two groups of controls was exam-

ined. The age, gender, and measured blood pressure distribution of the two control groups were nearly identical. The Health Family Tree group had significantly higher body mass index (BMI) ($28.6 \pm 5.5 \text{ kg/m}^2$ vs. $27.2 \pm 4.6 \text{ kg/m}^2$, $p < 0.0001$), plasma TG ($173 \pm 87 \text{ mg/dl}$ vs. $135 \pm 104 \text{ mg/dl}$, $p < 0.0001$), and glucose levels ($92.7 \pm 21.7 \text{ mg/dl}$ vs. $89.8 \pm 15.5 \text{ mg/dl}$, $p = 0.015$) and lower HDL-cholesterol ($47.5 \pm 13.2 \text{ mg/dl}$ vs. $53.0 \pm 16.1 \text{ mg/dl}$, $p < 0.0001$) and LDL-cholesterol ($103 \pm 28 \text{ mg/dl}$ vs. $109 \pm 28 \text{ mg/dl}$, $p = 0.0003$). Prevalence of having ever smoked daily for a year or more (18.8% vs. 26.1%, $p = 0.0050$) and current alcohol use (8.9% vs. 25.1%, $p < 0.0001$) were greater among the second group. A separate group of 498 Utah subjects recruited randomly from Health Family Tree families for a different study (the Family Heart Study; not used as control subjects in this study because lipids were not measured by ultracentrifugation) (25) was also examined for comparability with the two control groups. This comparison group was found to have intermediate levels of BMI ($28.2 \pm 5.6 \text{ kg/m}^2$) whereas HDL-cholesterol ($44.2 \pm 13.5 \text{ mg/dl}$) was more similar to the other Health Family Tree cohort and plasma TG levels ($139 \pm 78.4 \text{ mg/dl}$) were more similar to the control group from the psychology study. When compared with other general population groups from other sites in the Family Heart Study, the psychology study group was intermediate between these and the Utah Tree groups for the defined variables. Risk calculations were performed using each control group separately. In all instances, odds ratio estimates and trends were very similar and, in most categories, the odds ratio using one control group was included in the 95% confidence interval for the odds ratio using the other control group. Odds ratio estimates for type III hyperlipidemia, in particular, were similar using either control group. In a few HDL-TG subcategories (particularly those with TGs $>300 \text{ mg/dl}$), estimates of odds ratios were unstable (fewer than five control subjects) when the control groups were considered separately. Odds ratio estimates for LDL-cholesterol and non-lipid risk factors were also generally comparable. The two control groups were therefore combined for all further analyses. This study was approved by the Institutional Review Board of the University of Utah Medical Center. All subjects signed informed consent before participating.

A participant was considered to have hypertension if taking antihypertensive medication for a prior physician diagnosis of hypertension, or if at screening the mean of two sitting blood pressures taken with a Critikon Dynamap automated blood pressure machine was $\geq 140 \text{ mm Hg}$ systolic or 90 mm Hg diastolic. Diabetes was considered present if a prior physician diagnosis had been made or if the fasting serum glucose upon screening was $\geq 126 \text{ mg/dl}$. Cigarette smoking was dichotomized into "ever" or "never," with ever smoking defined as having smoked daily for one year or more. Many patients had quit after onset of their CAD, hence the designation as ever smoking rather than current and former.

Table 1. Clinical Characteristics

Variable	CAD Cases (n = 653)		Control Subjects (n = 1,029)		p Value
	Mean	SD	Mean	SD	
Age (yrs)	54.9	7.6	53.6	8.8	0.0017
Male (%)	76.4		47.4		<0.0001
BMI (kg/m ²)	28.6	4.9	27.9	5.1	0.0103
Hypertension (%)	58.3		27.2		<0.0001
Diabetes (%)	19.3		6.0		<0.0001
Ever smoked (%)	46.2		22.4		<0.0001
Alcohol use (% 1 + drink/week)	11.9		16.8		0.0048
Taking lipid-lowering medication (%)	27.6		9.1		<0.0001
Systolic BP (mm Hg)	136	21.1	121	17.4	<0.0001
Diastolic BP (mm Hg)	80.4	11.2	72.3	11.0	<0.0001
Glucose (mg/dl)	100.9	45.9	91.3	19.0	<0.0001
Albumin (mg/dl)	4.18	0.35	4.30	0.30	<0.0001
Bilirubin (mg/dl)	0.587	0.371	0.649	0.345	0.0006
Creatinine (mg/dl)	1.064	0.523	0.918	0.301	<0.0001
Uric acid (mg/dl)	6.26	1.51	5.59	1.49	<0.0001
Total cholesterol (mg/dl)	218	47	188	34	<0.0001
Triglycerides (mg/dl)	206	133	155	98	<0.0001
HDL-cholesterol (mg/dl)	40.6	12.0	50.2	15.0	<0.0001
LDL-cholesterol, measured (mg/dl)	136	42	106	28	<0.0001
VLDL-cholesterol, measured (mg/dl)	40.4	29.9	31.2	20.8	<0.0001
Type III hyperlipidemia (%)	3.4		0.98		0.0019

Cases with premature CAD are compared with population controls. Differences were tested by chi-square analysis or Student *t* test.

BMI = body mass index; BP = blood pressure; CAD = coronary artery disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.

Laboratory methods. Blood samples were collected in the morning after 12 h to 16 h of fasting and prepared according to guidelines of the Lipid Research Clinic's program *Manual of Laboratory Operations* (26). Lipid and lipoprotein concentrations were measured by a microscale ultracentrifugation procedure developed in our laboratory (27). We measured HDL-cholesterol by a magnetic bead precipitation using dextran sulfate/MgCl₂ (28). Cholesterol and TGs in total plasma and subfractions were measured with a Roche FARA II (Roche Diagnostics, Indianapolis, Indiana) automated analyzer. The TG-rich lipoproteins were separated from LDL and HDL by use of a Beckman TL100 ultracentrifuge and tube splicing. The value for very low-density lipoprotein (VLDL) cholesterol was taken as the measured cholesterol in the top fraction (density <1.006). This value was compared to the total cholesterol minus the cholesterol in the bottom fraction (density ≥1.006) containing LDL + HDL and verified to yield virtually identical results. Measured LDL cholesterol was taken as the cholesterol measured in this bottom fraction minus HDL-cholesterol. Type III hyperlipidemia was defined as present if the ratio of VLDL/plasma TGs was ≥0.30 and plasma TGs were >150 mg/dl (17). Our laboratory participates in the Centers for Disease Control Standardization Program and consistently achieves excellent agreement with Centers for Disease Control standards (coefficients of variation in the range of 0.5% to 3.3% for total cholesterol, TG, and HDL-cholesterol, with <3% difference in means).

Statistical analysis. The SAS Statistical Software Package (version 8.2) was used for data analysis (SAS Institute, Inc.

Cary, North Carolina). Statistical analyses on TGs were done after logarithmic transformation. Statistical tests included Student *t* test, chi-square, Pearson's correlation, and stepwise multiple logistic regression using SAS PROC LOGISTIC. Because some cases came from the same family, effects of subject relatedness were tested using PROC GENMOD in SAS. As results were nearly identical with the two methods, p values obtained with PROC LOGISTIC are reported.

RESULTS

Clinical characteristics of premature familial CAD cases are compared with control subjects in Table 1. Highly significant differences by univariate analysis were apparent for standard CAD risk factors as well as for several other measures including serum albumin, bilirubin, creatinine, and uric acid. Differences between cases and controls were similar to those shown in Table 1 for men and women considered separately and among those using or not using lipid-lowering medication (data not shown).

The risks of CAD across increasing strata of plasma TGs are shown in Table 2, excluding subjects with type III hyperlipidemia, who are considered separately. Because plasma HDL-cholesterol is inversely correlated with plasma TGs (*r* = -0.35 in cases and -0.40 in control subjects, both *p* < 0.0001) and because adjustment for HDL-cholesterol frequently diminishes associated risk of plasma TGs, models were tested with and without adjustment for HDL-cholesterol (and other TG-associated risk factors). In

Table 2. Odds Ratio From Multiple Logistic Regression for Triglyceride and HDL Categories

	N in Category (Cases, Control Subjects)	Minimally Adjusted Model*		Fully Adjusted Model*	
		OR (95% CI)	p Value	OR (95% CI)	p Value
Triglyceride categories					
<100 mg/dl	89, 284	1.0 (-)	—	1.0 (-)	—
100-149 mg/dl	171, 325	1.5 (1.04-2.1)	0.030	1.2 (0.81-1.8)	0.34
150-199 mg/dl	386, 420	1.8 (1.2-2.6)	0.0027	1.1 (0.70-1.7)	0.72
200-299 mg/dl	144, 150	1.9 (1.3-2.7)	0.0011	1.7 (1.1-2.6)	0.012
300-499 mg/dl	74, 49	3.8 (2.3-6.3)	<0.0001	2.8 (1.6-4.9)	0.0003
500-799 mg/dl	19, 7	14.8 (5.3-41.6)	<0.0001	11.4 (3.4-38.0)	<0.0001
800+ mg/dl	2, 1	33.0 (2.9-332)	0.0051	5.0 (0.29-85.1)	0.27
Type III hyperlipidemia	22, 10	5.2 (2.0-13.8)	0.0008	5.4 (1.9-15.4)	0.0016
HDL-cholesterol categories					
<25 mg/dl	30, 8	28.9 (11.1-75.3)	<0.0001	14.8 (4.8-45.4)	<0.0001
25-29 mg/dl	59, 32	7.2 (3.9-13.2)	<0.0001	5.1 (2.4-10.9)	<0.0001
30-34 mg/dl	123, 94	4.6 (2.9-7.4)	<0.0001	3.4 (1.9-6.4)	<0.0001
35-39 mg/dl	112, 134	2.7 (1.7-4.2)	<0.0001	2.0 (1.1-3.6)	0.015
40-49 mg/dl	174, 269	2.1 (1.4-3.1)	0.0003	1.9 (1.1-3.2)	0.013
50-59 mg/dl	89, 240	1.3 (0.82-1.9)	0.30	1.4 (0.85-2.5)	0.17
60+ mg/dl	37, 242	1.0 (-)	—	1.0 (-)	—

There were a total of 653 cases and 1,029 control subjects. In this analysis, patients with type III hyperlipidemia were not included in other triglyceride categories. *In the minimally adjusted models variables included were age, gender, cigarette smoking, and LDL-cholesterol besides the categories shown in the table. For the triglyceride category estimates, HDL was not included in the minimally adjusted model. For the HDL categories, triglycerides were not included in the minimally adjusted model. In the full model, independent variables were age, gender, smoking hypertension, diabetes, alcohol use (yes/no), measured LDL-cholesterol, albumin, bilirubin, creatinine and HDL-cholesterol as a continuous variable for the triglyceride categories or triglycerides as a continuous variable for the HDL categories.

CI = confidence interval; OR = odds ratio; other abbreviations as in Table 1.

addition, models for HDL-cholesterol-associated risk were also tested with and without similar adjustment for plasma TGs. Odds ratios for premature familial CAD were similar for men and women considered separately and are therefore shown with genders combined. Generally, TG-related risks were affected more by adjustment for HDL-cholesterol and other risk factors than HDL-associated risk was affected by adjustment for plasma TGs. Nevertheless, plasma TGs 200 mg/dl or above and type III hyperlipidemia remained significantly associated with premature familial CAD regardless of adjustment for HDL-cholesterol and other risk factors. Risk associated with lesser elevations of plasma TGs appeared to be more dependent on HDL-cholesterol.

There were 61 control subjects and 114 case subjects taking beta-blockers at the time of blood draws. Although TGs were 18.8 mg/dl (p = 0.04) higher in control subjects taking beta-blockers and 40.2 mg/dl higher in cases taking beta-blockers compared with nonusers in each group, risk estimates associated with TG categories shown in Table 2 were not materially affected by excluding those taking beta-blockers or by including use of beta-blockers as a covariate in multiple logistic models.

To further examine possible interactions between plasma TGs and HDL-cholesterol, combinations of different strata for each variable were modeled. Results of these analyses are shown in Table 3 and Figure 1. In this model, the estimated CAD risk associated with type III hyperlipidemia risk was even more elevated. Trends in CAD risk are apparent for increasing level of plasma TGs at all levels of HDL-cholesterol. For HDL-cholesterol there was a steep, statistically significant gradient of increasing risk associated with

decreasing levels within all TG strata, even when TGs were <100 mg/dl (data not shown for TGs <100 mg/dl).

We examined whether potential interactions among various features of the metabolic syndrome affected CAD risk prediction. Because waist circumference was not available for the premature familial CAD cases, a cut point of BMI ≥ 30 kg/m² was adopted as a surrogate marker for central obesity. Our definition of metabolic syndrome was otherwise the same as the Adult Treatment Panel III guidelines (29). In univariate analysis, the metabolic syndrome was associated with an odds ratio for premature familial CAD of 2.8 (95% confidence interval [CI] 2.2 to 3.4, p < 0.0001), which persisted after adjustment for age, gender, cigarette

Table 3. Odds Ratios for Different Combinations of Plasma Triglycerides and HDL-Cholesterol

Triglycerides	HDL Cholesterol	OR (95% CI)	p Value
Type III hyperlipidemia	—	9.7 (3.4-27.6)	<0.0001
<200 mg/dl	50+ mg/dl	1.0 (-)	—
200-299 mg/dl	50+ mg/dl	1.1 (0.45-2.8)	0.80
300+ mg/dl	50+ mg/dl	7.9 (1.1-54.0)	0.036
<200 mg/dl	40-49 mg/dl	1.3 (0.86-1.9)	0.22
200-299 mg/dl	40-49 mg/dl	3.7 (2.0-7.0)	<0.0001
300+ mg/dl	40-49 mg/dl	6.7 (2.4-19.0)	0.0003
<200 mg/dl	30-39 mg/dl	2.2 (1.4-3.4)	0.0003
200-299 mg/dl	30-39 mg/dl	3.1 (1.8-5.2)	<0.0001
300+ mg/dl	30-39 mg/dl	4.3 (2.1-8.6)	<0.0001
<200 mg/dl	<30 mg/dl	5.7 (2.4-13.1)	<0.0001
200-299 mg/dl	<30 mg/dl	6.1 (2.3-16.5)	0.0003
300+ mg/dl	<30 mg/dl	17.2 (7.2-41.2)	<0.0001

Independent variables in the model include all those in the fully adjusted model in Table 2. Odds ratios and p values are in reference to persons without type III hyperlipidemia with plasma triglycerides <200 mg/dl and HDL-cholesterol 50+ mg/dl.

Abbreviations as in Tables 1 and 2.

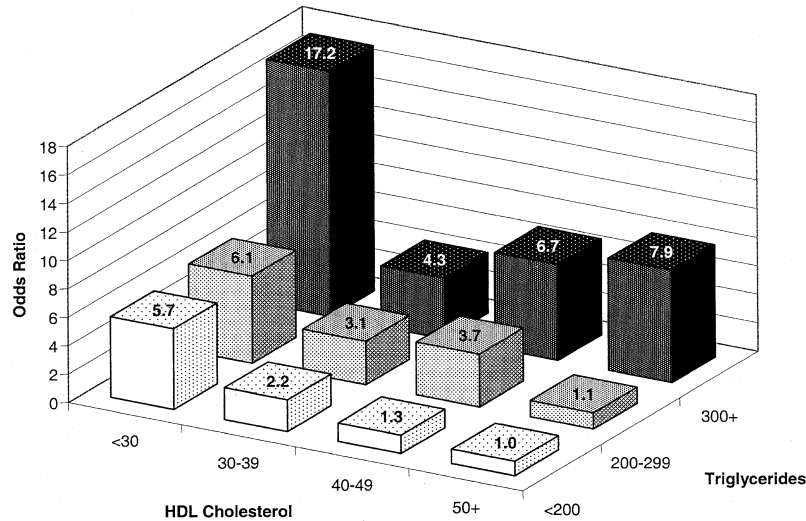


Figure 1. Odds ratios for premature coronary artery disease in mutually exclusive plasma triglyceride and high-density lipoprotein (HDL) categories. Confidence intervals and p values are given in Table 3.

smoking, and measured LDL-cholesterol in a multiple logistic model (odds ratio 3.0, 95% CI 2.3 to 3.8, $p < 0.0001$).

The degree of independence of the various components of the metabolic syndrome in predicting CAD risk remains unclear. We therefore examined intercorrelations between TGs and the other components of the metabolic syndrome. Prevalence and levels of each factor in both cases and controls increased as plasma TGs rose, as shown in Figure 2. Trends for these relationships were all highly significant among both case and control groups considered separately (most with $p < 0.0001$) (Fig. 2). Nevertheless, there was no evidence for interaction between plasma TGs and HDL-cholesterol, hypertension, or LDL cholesterol in predicting CAD risk; the contribution of plasma TGs to CAD risk remained independent. Although the prevalence of all the other features of the metabolic syndrome increased with increasing BMI, BMI was not independently associated with CAD risk (data not shown).

Because hypertension has been strongly implicated in the CAD risk associated with dyslipidemia in prior studies, we also examined hypertension status with high or low TGs and HDL-cholesterol (above and below 200 and 40 mg/dl, respectively) and found that hypertension added independently to CAD risk within each lipid category (Fig. 3).

Patients with premature familial CAD and type III hyperlipidemia were the same age at the time of screening as other CAD cases and had similar age at onset of CAD (50.1 ± 7.1 years vs. 47.8 ± 7.0 years, $p = 0.12$); they had similar BMI and gender distributions compared with other CAD cases. Compared with other CAD cases, type III cases had higher plasma total cholesterol (287 ± 60 mg/dl vs. 216 ± 45 mg/dl, $p < 0.0001$), TGs (268 ± 103 mg/dl vs. 203 ± 133 mg/dl, $p = 0.025$), and, as expected, much higher measured VLDL-cholesterol (100.0 ± 46.2 mg/dl vs. 38.4 ± 26.9 mg/dl, $p < 0.0001$), and by definition a higher ratio

of measured VLDL-cholesterol/plasma TGs (0.370 ± 0.065 mg/dl vs. 0.188 ± 0.047 mg/dl, $p < 0.0001$). Despite higher total cholesterol and TGs in type III cases, their measured LDL- and HDL-cholesterol were not different. Prevalence of diabetes, hypertension, and use of lipid-lowering medications and estrogen were also similar in type III versus other CAD cases.

Because other studies have utilized cut points of plasma TGs at 200 mg/dl and 800 mg/dl and HDL-cholesterol at 40 mg/dl, we also provide estimates of CAD risk for categories based on these cut points considering patients with type III hyperlipidemia as a separate category in the same model (Fig. 4). Risks in men and women considered separately were nearly identical except for TGs ≥ 800 mg/dl, for which estimated risk appeared much higher for women than men (though estimates for both genders had very wide confidence intervals and the differences were nonsignificant; data not shown).

DISCUSSION

Our principal finding was that plasma TGs predicted CAD risk independent of total cholesterol and HDL-cholesterol. The fact that CAD risk was increased substantially with TG elevations beginning at 200 mg/dl is of clinical significance because this is a common and relatively mild lipid abnormality. This is of even greater importance because the increase in CAD was seen even with HDL-cholesterol above 40 mg/dl. Furthermore, this risk was independent of other features of the metabolic syndrome. Risk of CAD was approximately doubled with either TGs ≥ 200 mg/dl or HDL-cholesterol < 40 mg/dl. The presence of both was associated with a four-fold increase in risk (Fig. 4).

Only one other published study has found such a strong and independent relationship between TGs and CAD. Total cholesterol, TGs, and HDL were each independent

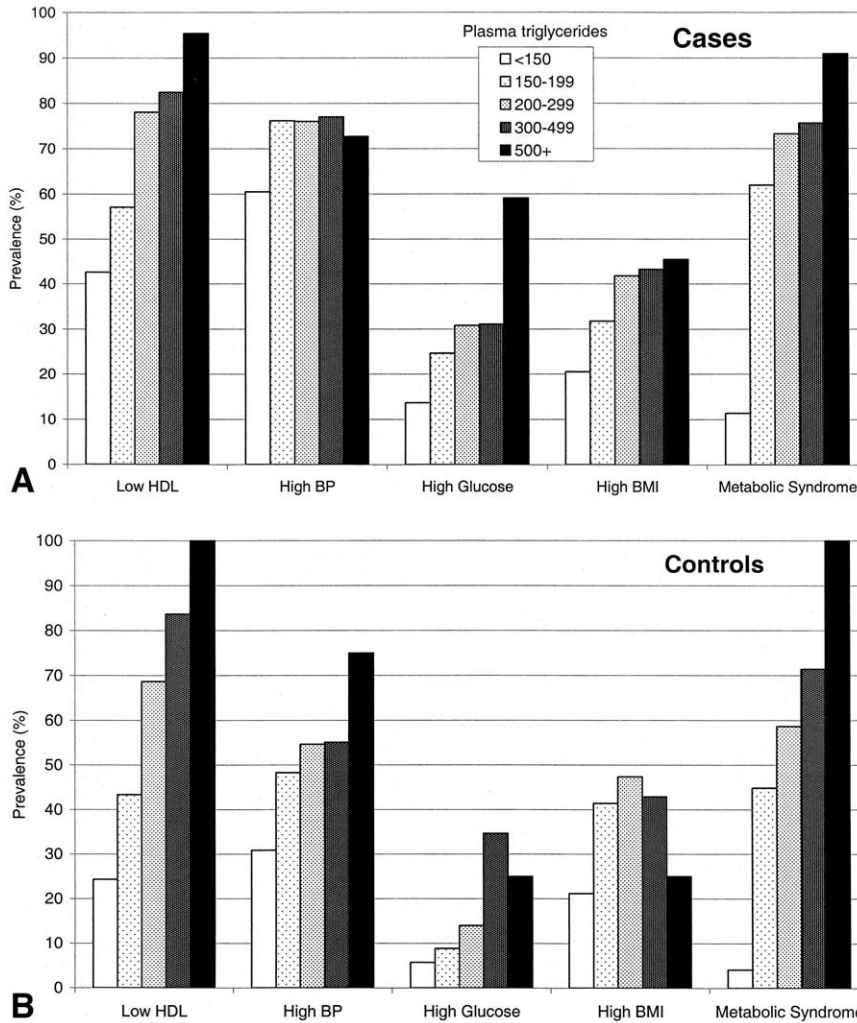


Figure 2. Prevalence of metabolic syndrome and contributing factors by plasma triglyceride concentration in cases (A) and control subjects (B). The legend shows triglyceride strata (mg/dl). Cut points for factors of the metabolic syndrome are as defined by ATP-III except body mass index (BMI) which for this study was defined as BMI ≥ 30 kg/m². All trends were significant with $p < 0.0001$ except for the high blood pressure (BP) in cases for which the p value for trend was 0.002. Patients with type III hyperlipidemia were excluded. HDL = high-density lipoprotein.

risk factors and CAD risk was predicted by a logistic regression model in United Kingdom men (30). In contrast, most other studies have failed to see such a strong independent association. For example, CAD risk increased with increasing TG in the Physician's Health Study and was independent of HDL-cholesterol, but was not independent of serum total cholesterol (31). More commonly, plasma TGs were reported to be a risk factor only when HDL-cholesterol was low (13,14). Others have reported interactions of the relationship between CAD and plasma TGs with total cholesterol as well as age (32,33). It is possible that the studies that found increased risk with high TGs only when HDL was low had insufficient power to detect an independent contribution of elevated TGs with higher HDL-cholesterol. This is because patients with both high TGs and high HDL-cholesterol are a relatively uncommon subgroup.

We found remarkably increased CAD risk for persons with plasma TGs in the 500 to 799 mg/dl range and we

found that this high risk persisted even with adjustment for HDL and other risk factors (odds ratio = 11.4, 95% CI 3.4 to 38.0). Although the confidence interval was relatively wide, the finding was highly significant ($p < 0.0001$) and essentially unaffected by eliminating users of beta-blockers or adjusting for use of beta-blockers in the multiple logistic models. This degree of excess risk might seem greater than the apparently modest risk associated with higher TGs in the meta-analyses of Hokanson and Austin (1,34). These investigators noted statistically significant relative risks of 1.32 and 1.76 for each 1 mmol/l (88.6 mg/dl) increase in TGs among men and women, respectively. Risks were diminished but remained significant with adjustment for HDL. Assuming a 50% increase in risk for each 1 mmol/l rise in TGs (approximate average from the above cited meta-analyses) (1,34) and given a 5 mmol/l increase from our lowest category to the 500 to 799 mg/dl range, one can calculate an expected relative risk of 7.6, which is well within the confidence interval we found for this category.

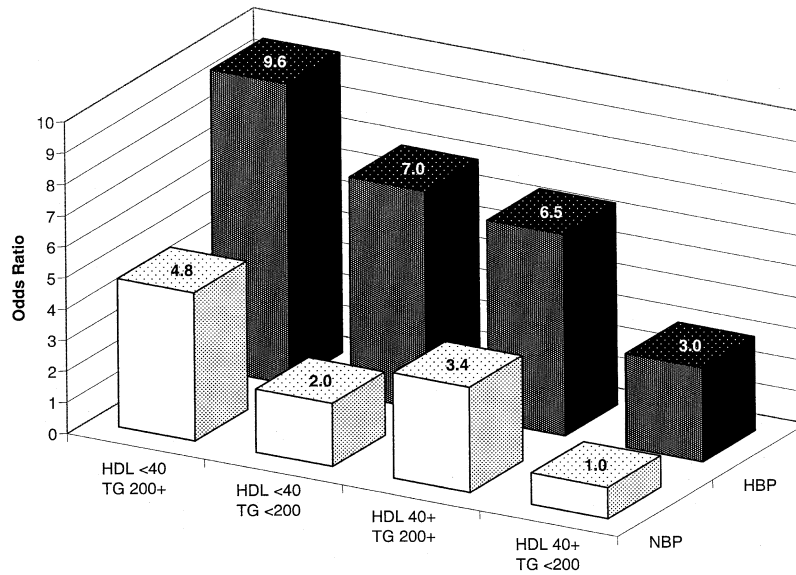


Figure 3. Odds ratios of premature coronary artery disease in triglyceride (TG)/high-density lipoprotein (HDL) categories and by hypertension. Odds ratios for normal blood pressure (NBP), high HDL, high TG and low HDL, low TG categories were significant at $p = 0.006$ and 0.0010 respectively. All others are significant at $p < 0.0001$. HBP = high blood pressure.

Most other studies have not included sufficient subjects with TGs in this range to have much statistical power to examine risk associated with TGs above 500 mg/dl.

Type III hyperlipidemia (also called familial dysbetalipoproteinemia) is defined by the accumulation in plasma of highly atherogenic, abnormal, cholesterol-enriched remnants of TG-rich lipoproteins (35). Although a high prevalence of premature atherosclerosis in coronary and other arteries is readily apparent from case series of patients with type III hyperlipidemia (18,36-42), no quantitative estimates of risk have previously been available. Our study is apparently the first to perform ultracentrifugation in all cases and controls and thereby identify type III hyperlipidemia by classic methods even in patients with mild hypertriglyceridemia. Surprisingly, 65.6% and 87.5% of patients we identified as having type III disease would not have been identified if we had restricted ultracentrifugation only to those with TGs ≥ 300 mg/dl or ≥ 400 mg/dl, respectively.

Our population-based calculations of risk of premature familial CAD associated with type III hyperlipidemia (odds ratio from 5 to 10 depending on the model) are consistent with rough estimates of risk based on the prevalence of type III in the general population and among premature CAD cases. In the Lipid Research Clinics Prevalence Study, type III hyperlipidemia was found in 0.4% of men in the general population (43). The somewhat higher prevalence of type III in our controls (10 of 1,019, or 0.97%) may possibly be due to the increase in the prevalence of obesity since the time when the Lipid Research Clinics study was performed, 18 years before our study. In the series of premature CAD cases identified by Goldstein et al. (44), type III was reported in 2.5% to 5% of 156 premature CAD cases with hyperlipidemia for whom ultracentrifugation was performed, similar to the prevalence in our cases (22 of 653, or

3.4%). Plasma lipid levels in type III hyperlipidemia can be highly variable, but levels found in our type III patients were similar to those reported by Goldstein et al. (44) in their type III patients with CAD.

Several measures of increased remnant accumulation are associated with elevated CAD risk. Unfortunately, estimates of the degree of risk elevation vary substantially, depending apparently on the method or parameter used (45-48). In contrast, little or no excess CAD risk has been reported by some investigators for those with hyperchylomicronemia or type V hyperlipidemia even though TG levels are much higher (15,16). The variability of risk with hypertriglyceridemia probably is related to the particular species of TG-rich particles present in excess. For example, in diabetic cholesterol-fed rabbits, particles >75 nm in diameter (including larger VLDL and chylomicrons) infiltrated the artery wall poorly if at all and therefore presumably made little direct contribution to atherogenesis (49). Unlike LDL-cholesterol, therefore, risk associated with total plasma TGs may not be appropriately modeled by a simple, monotonically increasing risk function. Rather, CAD risk from hypertriglyceridemia may be more accurately estimated by examination of specific TG-related disorders.

The CAD risks we found for other standard risk factors in this study are comparable to prior reports and generally support a multifactorial approach to CAD prevention (29). Our findings also corroborate findings regarding serum albumin (50,51) (inversely correlated with high sensitivity C-reactive protein) (52) and bilirubin (53-56).

There are several limitations to our study. As this is a case-control study, we cannot report on absolute risk associated with the various lipid levels nor can we demonstrate temporal sequence (i.e., that risk factors were present before the onset of CAD). There are, however, data from prospec-

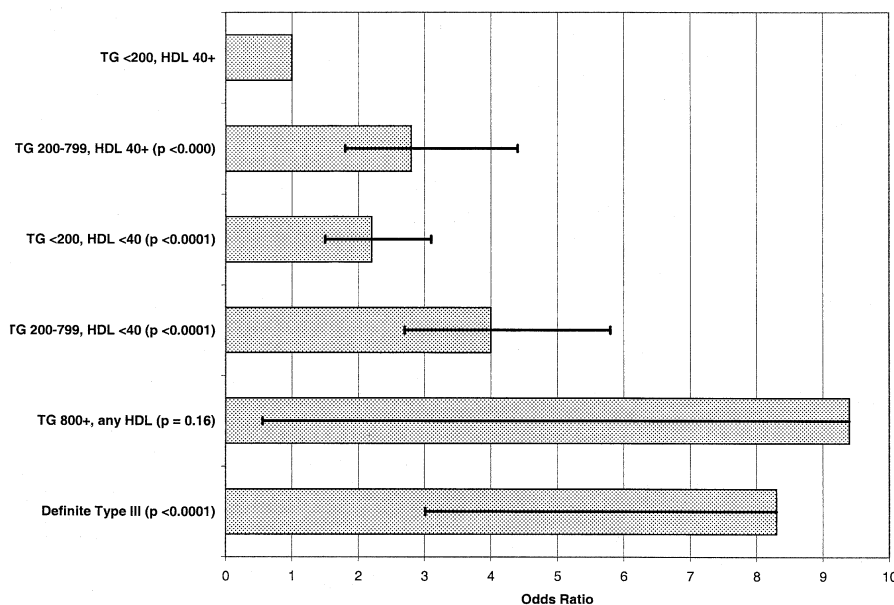


Figure 4. Summary of coronary artery disease risk (odds ratios and 95% confidence intervals) associated with commonly used cut points. Risks were estimated simultaneously in a multiple logistic regression model adjusting for age, gender, hypertension, diabetes, serum albumin, bilirubin, creatinine, and low-density lipoprotein cholesterol. HDL = high-density lipoprotein; TG = triglycerides.

tive studies demonstrating little long-term change (after one to two months post-infarct) in untreated lipids after the clinical diagnosis of CAD (57-59). A related limitation is the effect of differential intervention on lipids because, as expected, our CAD cases had more aggressive treatment than our control cases. Greater lipid intervention among CAD patients would result in lower LDL-cholesterol and TG levels and a resulting underestimation of the odds ratio associated with these lipids. Lipid interventions also tend to have a salutary, though generally smaller, effect on HDL-cholesterol. Therefore, estimates of risk reported in our study are likely to be conservative. Importantly, however, restricting analyses to those not taking lipid-lowering medication or inclusion of such therapy as an effect variable did not materially affect our results (data not shown). This may be due, in part, to the relative infrequency of lipid-lowering drug therapy in our premature familial CAD cases, reflecting usual clinical practices in the mid-1990s as reported in several surveys (60,61). Further, there were no significant differences in lipid levels among our cases comparing those taking with those not taking lipid-lowering medication (data not shown), possibly reflecting relatively ineffectual or inadequate treatment primarily restricted to patients with high untreated levels.

Hypertension was considerably more frequent among our cases than control subjects. We cannot exclude the possibility that some of the higher TGs and lower HDL-cholesterol seen in our cases may have been due to greater use of diuretics and beta-blockers. Recent studies of these agents suggest, however, that the long-term lipid effects of these agents are small, especially at the lower doses used predominately during the past 10 to 15 years (62-66). Furthermore, inclusion of hypertension as a term in the

multiple logistic regression model would help mitigate any confounding due to antihypertensive medications.

Potential limitations of case-control studies in general include recall bias and selection bias. Because all cases and controls were examined in our clinic with blood testing performed by a single laboratory, various forms of information bias were minimized. In particular, recall bias, a common problem in case-control studies as opposed to prospective studies, was minimized in this study because all subjects were interviewed in our clinic and cases were not asked to recall factors present before the onset of their CAD other than lifetime use of cigarettes. Inappropriate selection of cases and controls can inadvertently introduce bias into a case-control study. Sampled cases should be representative of all cases arising from a given population and controls should be representative of the general population. Our CAD cases were defined as premature and familial, thus potentially limiting generalization of our findings to non-familial or older onset CAD. Nevertheless, a large proportion of premature CAD cases do show evidence for a positive family history of CAD (67). As our premature familial CAD cases are the result of an intensive search for such cases among the major urban and suburban areas of Utah, we believe these to be representative of all such cases in Utah. As participants in the multicenter Family Heart Study (25) (with recruitment sites at Framingham, Minneapolis, Utah, and Forsyth County, North Carolina), we were able to directly compare our premature familial CAD cases and controls with premature CAD cases and general population controls in the Family Heart Study (19). The definitions of premature CAD in the Family Heart Study were identical to our current study, though not all cases in the Family Heart Study had positive family histories of

premature CAD. Compared with CAD cases in the Family Heart Study, our cases were about five years younger with a slightly higher percentage of men, higher blood pressure, and substantially lower alcohol use. Other risk factors and lipids in our CAD cases were similar to CAD patients at other sites in the Family Heart Study, particularly after controlling for alcohol use (data not shown). Only living CAD patients could have participated in our study. As survivors of clinically apparent CAD could represent less severely affected patients as compared with those who had died, bias may be introduced that would diminish odds ratios (as more severe CAD would be expected to have more severe risk factors). This limitation is common to all case-control studies of CAD.

The selection of an appropriate control group is also critical to the validity of a case-control study. One of our control groups was randomly selected from a general population (Health Family Tree Program), which on close evaluation represented largely suburban families with very low use of alcohol and prevalence of ever smoking. The second control group, though not randomly selected, represented primarily urban couples. Comparison of these control groups with control groups from the Family Heart Study (19) again showed much lower smoking rates and alcohol use in the controls from the Health Family Tree Program with more intermediate levels in the urban couples. Other risk factors were comparable with controls at other Family Heart Study sites. Combining the control groups therefore provides a wider representation of the general Utah population (both urban and suburban). The very similar estimates of risk using each of these control groups in separate analyses further justifies combining the two control groups and provides strong evidence for the validity of our findings.

In conclusion, we found a strongly increased risk for premature familial CAD with elevated plasma TGs. Importantly, this excess risk begins at a mild and relatively common TG elevation of just 200 mg/dl, and is independent of plasma HDL-cholesterol, other elements of the metabolic syndrome, and other CAD risk factors. In addition, for the first time we provide a quantitative, population-based estimate of the markedly elevated risk of CAD associated with type III hyperlipidemia determined by ultracentrifugation.

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