Homocysteine and Lipoprotein(a) Interact to Increase CAD Risk in Young Men and Women

JoAnne Micale Foody, John A. Milberg, Killian Robinson, Gregory L. Pearce, Donald W. Jacobsen, Dennis L. Sprecher

Abstract—A biochemical link between homocysteine (tHcy) and lipoprotein(a) [Lp(a)] related to fibrin binding has been proposed. This hypothesis has not been specifically examined in human subjects. We sought to determine in a clinical setting whether these risk factors would interact to increase coronary artery disease (CAD) risk. We performed a cross-sectional analysis of 750 men and 403 women referred to a preventive cardiology clinic at the Cleveland Clinic Foundation, in whom baseline tHcy and Lp(a) data were available. Logistic regression after adjusting for standard cardiovascular risk factors was used to estimate the relative risk of CAD in patients with an Lp(a) ≥30 mg/dL and a tHcy ≥17 μmol/L. Neither isolated high tHcy (odds ratio [OR]=1.06, P=0.89) nor isolated high Lp(a) (OR=1.15, P=0.60) appeared to be associated with CAD in women. However, strong evidence of an association was seen when both risk factors were present (OR=4.83, P=0.003). Moreover, this increased risk showed evidence of an interactive effect beyond that attributable to either additive or multiplicative effects of tHcy and Lp(a) (P<0.003). In contrast, both elevated tHcy (OR=1.93, P=0.05) and elevated Lp(a) (OR=1.87, P=0.01) showed evidence of being independent risk factors for CAD in men. The presence of both risk factors in men did not appear to confer additional risk (OR=2.00, P=0.09), even though ORs as high as 12.4 were observed within specific age intervals. Consistent with prior studies, tHcy and Lp(a) are risk factors, either independently or in concert, for CAD in this clinical population. More significantly, we found evidence that when both risk factors were present in women, the associated risk was greater than what would be expected if the 2 risks were simply acting independently. The absence of such an interactive effect in men may be due to the confounding effects of age manifested as “survivor bias.” These clinical findings provide insights into the potential roles of both tHcy and Lp(a) in the pathogenesis of atherosclerosis.

Key Words: lipoprotein(a) ■ homocysteine ■ coronary artery disease ■ risk factors

Lipoprotein(a) [Lp(a)] and homocysteine (tHcy) are 2 important independent risk factors for coronary artery disease (CAD).1–18 Each may play an important role in the development of atherosclerosis through effects on thrombolysis, the endothelium, and platelets, yet the exact mechanisms by which they exert their pathogenicity have not been clearly defined. It has been suggested that tHcy may modulate the toxicity of Lp(a) via binding to plasmin-modified fibrin.19 No specific analyses have been performed to determine whether these 2 risk factors interact in a clinical population.

Lp(a), an LDL particle with the apo(a) protein attached by a disulfide bridge, is found to be elevated in approximately one third of CAD patients.20 The apo(a) moiety has structural similarities to plasminogen. Thus, with its lipid and plasminogen-like component, Lp(a) may provide a link between atherothrombosis and atherosclerosis. The evidence that Lp(a) is a CAD risk factor is derived from multiple epidemiological and clinical studies.3–6,20–25 tHcy, a metabolic product of the amino acid methionine, has also been linked to atherosclerosis.1–8

Elevated Lp(a) and an elevated tHcy appear to increase CAD risk even more in the presence of other risk factors. Lp(a) is particularly important in men in whom LDL cholesterol (LDL-C) is elevated.9 Graham et al11 have shown that tHcy potentiated the risk associated with hypertension, hypercholesterolemia, and smoking, whereas Ridker et al10 have shown an interaction between tHcy and factor V Leiden. These reported interactions of Lp(a) and tHcy with other cardiac risk factors, as well as the postulated biochemical link between the 2,11 may suggest a clinically relevant interaction between Lp(a) and tHcy.

To the best of our knowledge, no prior study has specifically addressed whether Lp(a) and tHcy interact in a general clinical population, and few data are available on the joint effect of these 2 risk factors in male and female subjects. We examined data from men and women in a large, preventive...
cardiology clinic to determine whether patients exposed jointly to high \( \text{Lp(a)} \) and \( \text{tHcy} \) were at increased risk of CAD compared with patients with only 1 or neither risk factor elevated.

**Methods**

We performed a cross-sectional study of baseline characteristics of all patients referred to the Cleveland Clinic Foundation (CCF) Preventive Cardiology and Rehabilitation Program (PCRP) from 1996 through March 1998 and included those with both a measured \( \text{Lp(a)} \) and \( \text{tHcy} \) for analysis (\( N=1153 \)). Mean ages for men and women were 56.2±12.77 and 57.7±12.46 years, respectively. Patients were routinely assessed for traditional cardiovascular risk factors as well as \( \text{tHcy} \) and \( \text{Lp(a)} \). Patients with known CAD were referred to the clinic as well as those without known CAD but who were considered high risk (non-CAD control group).

The PCRP receives patient referrals from an already high-risk, tertiary care referral population and seeks to reduce the risk of a first cardiac event or its recurrence (primary and secondary prevention). Cases and controls were referred at the discretion of their primary care internist, cardiologist, or cardiac surgeon. No patient was specifically referred because of an elevated \( \text{tHcy} \) or \( \text{Lp(a)} \). \( \text{tHcy} \) and \( \text{Lp(a)} \) were determined after initial consultation.

**Cases**

The majority of patients with a history of CAD were referred from cardiologists or internists at CCF. One third of our cohort was referred from outside the hospital. Demographic, general medical, and cardiovascular disease history are initially obtained by patient self-report. CAD was diagnosed in the presence of (1) a documented myocardial infarction; (2) a stenosis of ≥70% of at least 1 epicardial coronary vessel, as documented at the time of coronary angiography carried out in a standard manner; (3) coronary artery bypass grafting; or (4) an abnormal cardiac function test. Of those referred, 58.89% (\( n=445 \)) were documented to have CAD; 27.8% were post–myocardial infarction cases, 23.2% were post–percutaneous transluminal coronary angioplasty or other percutaneous intervention cases, and 27.6% were post–coronary artery bypass graft cases. Self-reported cardiovascular disease history was validated via the CCF Cardiovascular Interventionsal Registry and the PCRP patient registry for 1996 and 1997. In a subset of 370 patients with CAD, we verified surgical interventions and/or catheterization reports in some patients (64%) classified as having CAD. These patients had undergone either a coronary bypass operation, angioplasty, or a cardiac catheterization, which revealed a ≥50% occlusion in at least 1 major coronary artery. The remaining patients classified with CAD in 1996 to 1997 were referred from outside institutions or physicians. The presence of CAD in these cases was determined by either medical chart review or patient self-report.

**Controls**

Controls were patients without known CAD who had been referred to the prevention clinic for various reasons, including high cholesterol levels, hypertension, obesity, smoking habits, and/or a family history of CAD. Cardiovascular diagnostic information, when available (eg, catheterization reports or functional tests), was evaluated to rule out CAD in these patients. Seventy-five percent of cases and a similar proportion of the controls reside in Cuyahoga County in northeastern Ohio; an additional 15% are from other parts of the state, while the remaining 10% are split between domestic and international locations. Eight percent of the population were nonwhite, and overall results were not impacted by their inclusion or exclusion.

**Data Collection**

All patients (cases and controls) were seen in the PCRP Clinic and underwent a standard clinical examination by nurses and physicians, which included anthropometry (height, weight, waist-hip ratio), blood pressure, and a blood draw for a lipid profile. Height was measured by using a stadiometer. Patients also received dietary and smoking counseling when necessary. Although patients were typically followed up every 3 to 6 months, measurements for this analysis were taken from the baseline evaluation only. Individuals also completed a questionnaire that incorporated numerous risk-related issues, including a history of hypertension, family history, cholesterol medication use, and diabetes (ever treated or diagnosed by a physician), and, in women, menopausal status and use of hormones. Patients were classified as either never- or ever-smokers. Hypertension was defined as a blood pressure >140/90 mm Hg, a history of hypertension, or the use of antihypertensive medications. Diabetes mellitus was diagnosed if the patient was using insulin or an oral hypoglycemic agent or reported a history of diabetes mellitus.

**Biochemical Analyses**

Fasting total cholesterol, HDL-C, LDL-C, and triglyceride concentrations in the blood sample drawn at the same time as the sample for measurement of \( \text{tHcy} \) and \( \text{Lp(a)} \) were measured in all subjects. Lipoproteins were measured in serum after a 12-hour fast in our hospital-based Centers for Disease Control and Prevention–standardized laboratory and according to Lipid Research Clinics methodology. All samples with triglyceride values >400 mg/dL were analyzed by \( \beta \)-quantification.

**tHcy Assay**

Total fasting serum \( \text{tHcy} \) was measured on samples drawn on initial consultation at the PCRP at the CCF by use of high-performance liquid chromatography, as previously reported by Jacobsen et al. Serum \( \text{tHcy} \) has been shown to be \( \sim 10\% \) to \( 30\% \) higher than plasma \( \text{tHcy} \). The normal range in our laboratory is 0 to 15 \( \mu \text{mol/L} \), the mean value is 7 \( \mu \text{mol/L} \) and the SE is 2 \( \mu \text{mol/L} \). A \( \text{tHcy} \) cutoff of 17 \( \mu \text{mol/L} \) was used for all initial interaction analyses, which is the 90th percentile of \( \text{tHcy} \) values obtained in our clinic.

**Lp(a) Assay**

\( \text{Lp(a)} \) measurements were performed in our hospital chemistry laboratory by using standard methods and reagents (Incatr Co) according to the supplier’s package insert. In brief, this is an automated immunoprecipitation procedure that uses a monospecific goat antibody to \( \text{Lp(a)} \). The coefficient of variation is 13% for \( \text{Lp(a)} \) levels <10 mg/dL and 2.5% for \( \text{Lp(a)} \) levels >60 mg/dL. Values <5 mg/dL are undetectable. Short-term post–myocardial infarction increases in \( \text{Lp(a)} \), believed to be only transient, return to normal levels within 1 month. In our patients, the median time between a coronary event and presentation to our clinic was 10 months.

**Statistical Analysis**

**Analysis of Interaction**

Interaction between 2 exposures implies that the effect on disease risk when the 2 risk factors are present exceeds that which is expected based on their independent effects alone. On an additive scale, the 2 risk factors are added together (minus the baseline of exposure to neither) to estimate their combined effect, and on a multiplicative scale, they are multiplied together. If no interaction is present, then the joint effect of 2 exposures will simply be equivalent to these independent risks added or multiplied together. We used an \( \text{SAS} \) program (C. Daskalakis, PhD, Department of Biostatistics, Harvard School of Public Health, Boston, Mass) (Daskalakis and Lipsitz, unpublished data, 1999 and Reference 34) to test (1) whether a regression model that included a term for interaction between the main exposure variables was superior to a model that ignored the interaction and (2) whether the presence of the interaction was significant compared with a model that assumed no interaction on either an additive or multiplicative scale. Interaction in this context refers to the “attributable proportion,” or the amount of disease caused by nonadditivity among subjects with both risk factors. Wald \( \chi^2 \) tests were used to test the hypothesis that nonadditivity was equal to zero as:

\[
\text{H_0: } (\Psi_{LM} - \Psi_{L} - \Psi_{M} + 1)/\Psi_{LM} = \psi &= 0, \text{ where } \\
\Psi_{LM} &= \text{odds ratio for high } \text{Lp(a)} \text{ and non-elevated } \text{tHcy}, \\
\Psi_{L} &= \text{odds ratio for non-elevated } \text{Lp(a)} \text{ and high } \text{tHcy}, \text{ and } \\
\Psi_{M} &= \text{odds ratio for high } \text{Lp(a)} \text{ and high } \text{tHcy}.
\]
TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-CAD</th>
<th>CAD</th>
<th>P</th>
<th>Non-CAD</th>
<th>CAD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%), Males</td>
<td>255 (34)</td>
<td>495 (66)</td>
<td>0.0001</td>
<td>228 (56)</td>
<td>175 (44)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age, y (SD), Males</td>
<td>49 (11)</td>
<td>60 (11)</td>
<td>0.0001</td>
<td>55 (12)</td>
<td>62 (11)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age &lt;55 y, n (%), Males</td>
<td>176 (69)</td>
<td>162 (33)</td>
<td>0.001</td>
<td>114 (50)</td>
<td>45 (26)</td>
<td>0.001</td>
</tr>
<tr>
<td>Race, % black, Males</td>
<td>6.7</td>
<td>5.6</td>
<td>0.58</td>
<td>10.0</td>
<td>21.7</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI, kg/m² (SD), Males</td>
<td>30.7 (15)</td>
<td>29.6 (12)</td>
<td>0.29</td>
<td>29.4 (13)</td>
<td>32.0 (19.9)</td>
<td>0.06</td>
</tr>
<tr>
<td>Systolic BP, mm Hg, Males</td>
<td>130 (18)</td>
<td>133 (20)</td>
<td>0.06</td>
<td>135 (23)</td>
<td>140 (26)</td>
<td>0.11</td>
</tr>
<tr>
<td>Lipid-lowering drug, %, Males</td>
<td>32.5</td>
<td>56.1</td>
<td>0.001</td>
<td>31.4</td>
<td>57.2</td>
<td>0.001</td>
</tr>
<tr>
<td>History of smoking, %, Males</td>
<td>46.7</td>
<td>69.1</td>
<td>0.001</td>
<td>41.0</td>
<td>62.9</td>
<td>0.001</td>
</tr>
<tr>
<td>History of HTN, %, Males</td>
<td>33.2</td>
<td>53.9</td>
<td>0.001</td>
<td>41.4</td>
<td>28.6</td>
<td>0.001</td>
</tr>
<tr>
<td>History of diabetes, %, Males</td>
<td>9.8</td>
<td>17.6</td>
<td>0.001</td>
<td>8.8</td>
<td>52.0</td>
<td>0.001</td>
</tr>
<tr>
<td>n (%), Females</td>
<td>245 (34)</td>
<td>495 (66)</td>
<td>0.0001</td>
<td>228 (56)</td>
<td>175 (44)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age, y (SD), Females</td>
<td>49 (11)</td>
<td>60 (11)</td>
<td>0.0001</td>
<td>55 (12)</td>
<td>62 (11)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age &lt;55 y, n (%), Females</td>
<td>176 (69)</td>
<td>162 (33)</td>
<td>0.001</td>
<td>114 (50)</td>
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<tr>
<td>Race, % black, Females</td>
<td>6.7</td>
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<td>History of HTN, %, Females</td>
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<td>53.9</td>
<td>0.001</td>
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<td>0.001</td>
<td>8.8</td>
<td>52.0</td>
<td>0.001</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; BP, blood pressure; and HTN, hypertension.

Selection of Cutpoints

In our primary analysis, we used preestablished, dichotomous cutpoints for our main exposures. An Lp(a) level ≥30 mg/dL was considered elevated. This value has been used in a number of prior epidemiological studies20,23,32,35,36 and is the 70th percentile in our non-CAD population. This cutpoint was determined a priori and is the clinical cutpoint used in our clinic to determine elevated risk secondary to hyperhomocysteinemia. For tHcy, values ≥17 µmol/L were considered high. This is based on our hospital laboratory’s established upper limit of normal and is 2 SDs above the mean value of all tHcy values in males referred to our clinic. It is also the 90th percentile in our population. This value was determined a priori and is the clinical cutpoint used in our clinic to determine an elevated risk secondary to hyperhomocysteinemia.

The joint effect of elevated Lp(a) and tHcy on the risk of CAD was evaluated in all patients with both measures available. For all analyses, we adjusted for the following additional CAD risk factors: age, race (black versus white), history of smoking (ever versus never), diabetes, LDL-C, HDL-C, triglycerides, use of lipid-lowering medication (yes versus no), and body mass index. In women, we considered elevated. This value has been used in a number of prior epidemiological studies20,23,32,35,36 and is the 70th percentile in our non-CAD population. This cutpoint was determined a priori and is the clinical cutpoint used in our clinic to determine elevated risk secondary to hyperhomocysteinemia.

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Results

Comparison of the main demographic and clinical features of our population shows that CAD cases were predominantly male (74%) and, in both sexes, 8 to 9 years older than their non-CAD counterparts (Table 1). Similarly, in males and females, CAD cases more often reported smoking, hypertension, and diabetes. In females, a history of diabetes was reported nearly 4 times more frequently in CAD cases than controls (26% versus 7%).

Slightly more than half of the subjects (58%) were documented to have CAD. This was recorded in the presence of 1 or more of the following criteria: (1) a documented myocardial infarction (27.8%, n=228); (2) a stenosis of ≥50% of at least 1 epicardial coronary vessel, as documented at the time of coronary angiography carried out in a standard manner (48%, n=385); (3) coronary revascularization procedure, including coronary artery bypass grafting (27.6%, n=226), percutaneous transluminal coronary angioplasty or stent (23.2%, n=190); or (4) an abnormal cardiac function test (18%).

Non-CAD controls were those patients without known coronary disease who were referred to our clinic for risk factor modification, principally hyperlipidemia and/or hypertension. In general, they had significantly higher levels of total cholesterol, LDL-C, and triglycerides (all P<0.01), reflecting the referral of high-risk patients to our clinic (Table 2) and the fact that a higher proportion of CAD patients were taking lipid-lowering medications at entry (53% versus 33%, P<0.01) At baseline, both CAD and non-CAD patients reported lipid-lowering medication use. However, even when patients taking lipid-lowering medications were excluded from the dataset, total cholesterol, LDL-C, and triglycerides were still higher in the non-CAD group (data not shown). HDL-C values were lower in both male and female CAD patients.

Homocysteine

tHcy was somewhat higher in men, although the percentage of men and women with elevated tHcy and CAD was similar (20.8% and 22.9%, respectively; Table 2). tHcy was a borderline risk factor for CAD in the total population (odds ratio [OR]=1.49, confidence interval [CI]=0.90 to 2.45; P=0.12). The association between tHcy and CAD was stronger in men (OR=1.93, CI=1.00 to 3.72; P=0.05) than in women (OR=1.06, CI=0.46 to 2.47; P=0.89).

Lipoprotein(a)

Table 2 shows that women generally exhibited higher Lp(a) levels than did men. Moreover, 51% of women with CAD compared with 39% of men with CAD had elevated Lp(a). Women, and particularly those with CAD, had the highest median Lp(a) values. Fifty-one percent of females with CAD had an Lp(a) >30 mg/dL compared with 39% of males with CAD (Table 2). The overall percent distribution of Lp(a) levels in CAD cases and controls shows the expected non-normal left-skewed distribution of this lipoprotein and the generally higher values among CAD patients. Lp(a) was an independent risk factor for CAD in men (OR=1.87, CI=1.21 to 2.91; P=0.01). In women, however, Lp(a) was not identified as a significant risk factor for CAD (OR=1.15, CI=0.58 to 2.47; P=0.60).

Interaction Results

Neither isolated high tHcy nor isolated high Lp(a) was identified as an independent risk factor in women. However, the conjoint presence of elevated tHcy and elevated Lp(a) indicated increased risk of CAD in women (OR=4.83,
Moreover, the magnitude of this effect was in excess of what would be expected if the risk factors were operating either additively or multiplicatively with regard to CAD risk. With the estimated ORs for either isolated high Lp(a) or high tHcy, the situation of multiplicative interaction would yield an expected combined hazard of 2.3 for the overall population, 3.6 for men and 1.2 for women. For women, the observed OR of 4.8 exceeds the expected OR (P = 0.03). In contrast, the combined elevations of both risk factors in men showed only marginal risk (OR = 2.0, CI = 0.90 to 4.44; P = 0.09).

To address the issue of potential confounding effects of male age with regard to tHcy and/or Lp(a) in the form of “survivor bias,” the a priori age cutpoint of 55 was used to define “young” patients. That cutpoint was moved upwards iteratively to determine whether the effects of these risk factors diminished with age. Figure 1 shows that the OR for the conjoint effect of high tHcy and high Lp(a) in men peaked when a cutpoint of 60 years was used. The conjoint effect then dropped as older patients were included with the progression of age cutpoints. Despite the wide CIs for the combined group, this pattern of results suggests that the interaction probably is relevant in men, even though the effects of these risk factors are confounded by age. There was no evidence that the results were confounded by age in women. In fact, in women this interaction strengthened with age (Figure 2).

### Discussion
This cross-sectional analysis demonstrates that Lp(a) and tHcy interact to increase the risk of CAD in women. Elevations of both factors increase the risk for the presence of CAD.

### TABLE 2. Median Plasma Lipid Levels at Baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>P, CAD vs. Non-CAD</th>
<th>Females</th>
<th>P, CAD vs. Non-CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-CAD</td>
<td>CAD</td>
<td></td>
<td>Non-CAD</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>237</td>
<td>210*</td>
<td>0.001</td>
<td>262</td>
</tr>
<tr>
<td>Mean</td>
<td>210–283</td>
<td>180–243</td>
<td>0.68</td>
<td>227–300</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>37</td>
<td>37</td>
<td>0.0001</td>
<td>48</td>
</tr>
<tr>
<td>Mean</td>
<td>29–44</td>
<td>31–44</td>
<td>0.58</td>
<td>41–59</td>
</tr>
<tr>
<td>Total cholesterol/HDL</td>
<td>6.9</td>
<td>5.8</td>
<td>0.0001</td>
<td>5.5</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>147</td>
<td>129*</td>
<td>0.0001</td>
<td>159</td>
</tr>
<tr>
<td>Mean</td>
<td>115–177</td>
<td>103–157</td>
<td>0.004</td>
<td>129–199</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>13</td>
<td>21*</td>
<td>0.004</td>
<td>23</td>
</tr>
<tr>
<td>Mean</td>
<td>4–34</td>
<td>6–42</td>
<td>0.005</td>
<td>5–47</td>
</tr>
<tr>
<td>Lp(a) ≥ 30 mg/dL, %</td>
<td>28.6</td>
<td>38.9</td>
<td>0.02</td>
<td>41.5</td>
</tr>
<tr>
<td>Mean</td>
<td>12.7</td>
<td>13.5*</td>
<td>0.002</td>
<td>11.0</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>10.9–15.1</td>
<td>10.9–16.3</td>
<td>0.01</td>
<td>8.9–14.1</td>
</tr>
<tr>
<td>Mean</td>
<td>10.9–15.1</td>
<td>10.9–16.3</td>
<td>0.01</td>
<td>8.9–14.1</td>
</tr>
</tbody>
</table>

*P<0.05, CAD vs non-CAD, †P<0.01.

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**Figure 1.** Aging and tHcy: Lp(a) effects in men. Bar graph demonstrates OR based on age for each group of high tHcy, high Lp(a), and both.

**Figure 2.** Aging and tHcy: Lp(a) effects in women. Bar graph demonstrates OR based on age for each group of high tHcy, high Lp(a), and both.
by nearly 5 times compared with a more modest risk associated with each variable individually. We determined that the risk associated with combined elevations of these 2 entities is in excess of what would be expected if the independent contributions of each of the 2 risk factors were added together ($P=0.03$).

The majority of retrospective case-control studies have found an association between high Lp(a) values and CAD, as have equivalent studies for tHcy. Prospective studies, in contrast, are contradictory, revealing both positive [Lp(a)] and negative [Lp(a)] and tHcy associations. For example, whereas both the Lipid Research Clinics follow-up trial and the Framingham Offspring cohort revealed a significant risk of CAD for an Lp(a) value $>30$ mg/dL, the Physicians’ Health Study did not show any significant association between Lp(a) and cardiac events. In parallel fashion, the Tromso study, 1 of the longitudinally followed cohorts, revealed a 40% increase in the risk of incident myocardial infarction associated with a 4-$\mu$mol/L increase in tHcy, although reanalysis of the Physicians’ Health Study data and initial findings of the Atherosclerosis Risk In Communities trial revealed no such associations.2

Our data support a number of cross-sectional studies showing an increased CAD risk with elevated Lp(a) levels. Previous studies on Lp(a) alone have reported stronger risks in younger men and a diminishing effect of Lp(a) on CAD risk in men $>55$ and 65 years of age. This age-specific pattern may reflect a survivor bias, whereby young men with elevated Lp(a) levels are at high risk of CAD-related mortality, but other CAD risk factors may predominate in the older men. In the current study, the effects of isolated high tHcy, isolated high Lp(a), and the conjoint presence of both risk factors diminished as progressively older men were included in models predicting CAD (Figure 2). These results are concordant with the results from the study by (1) Ngan et al., in which in young men, the hazard ratios increased from 1.1 to 1.9 in parallel with the density of the pre-β electrophoretic bands [representative of Lp(a) serum levels]; (2) positive findings in the Framingham Offspring cohort in men $<55$ years of age; and (3) the lack of an effect in the Physicians’ Health Study of men with a mean age of 59 years. Thus, an interaction between Lp(a) and tHcy in men similar to that observed in women is likely, although potentially confounded in these current analyses by advancing age.

In women, the value of Lp(a) for predicting CAD has also been inconsistent. In a recent population-based study of both premenopausal and postmenopausal women, a significant increase in CAD risk (OR=2.9) was observed in those with Lp(a) levels $>30$ mg/dL versus those in the lowest quartile of their population ($<6$ mg/dL). This was consistent with the 14-year follow-up of nearly 5000 women in a recent Mayo Clinic study (hazard ratio=1.9), as well as a past study in young women from Framingham. In contrast, analyses of 2 prospective studies, the Stanford Five City Project and a Japanese trial of 337 women with population ages comparable to our own cohort, identified no significant association. Although we found a marginal association between Lp(a) and CAD in women overall, a significant and profound association was observed only when the elevation in Lp(a) was introduced. In contrast, in a prospective analysis of young women from Framingham, in young men, the value of Lp(a) for predicting CAD has also been inconsistent. In a recent population-based study of both premenopausal and postmenopausal women, a significant increase in CAD risk (OR=2.9) was observed in those with Lp(a) levels $>30$ mg/dL versus those in the lowest quartile of their population ($<6$ mg/dL). This was consistent with the 14-year follow-up of nearly 5000 women in a recent Mayo Clinic study (hazard ratio=1.9), as well as a past study in young women from Framingham. In contrast, analyses of 2 prospective studies, the Stanford Five City Project and a Japanese trial of 337 women with population ages comparable to our own cohort, identified no significant association. Although we found a marginal association between Lp(a) and CAD in women overall, a significant and profound association was observed only when the elevation in Lp(a) was accompanied by a concurrent elevation of serum tHcy levels.

There are 2 studies that address the interaction between Lp(a) and tHcy. Hopkins et al. analyzed plasma Lp(a), lipids, and other coronary risk factors in a case-control study of men and women with premature atherosclerosis. The relative risk for CAD in patients with Lp(a) values $>40$ mg/dL was 2.9. There was suggestive evidence for an interaction between Lp(a) and nonlipid risk factors, especially tHcy, even though a test for the significance of the interaction per se was not presented. An Lp(a) level $>40$ mg/dL and high tHcy resulted in an OR of 32, with a CI between 6.5 and 155 ($P=0.00002$). No sex-based analysis of the interaction was introduced. In contrast, in a prospective analysis of young men with premature peripheral atherosclerosis (N=95), Valentine et al. compared 50 white men aged 45 or younger at the onset of symptoms with age- and race-matched controls. Atherosclerotic risk factors were similar in both groups. These investigators reported no significant interaction be-

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**Figure 3. Proposed mechanism for Lp(a)/tHcy interaction.** As demonstrated in vitro, tHcy may dissociate apo(a) from Lp(a) in vivo, thereby exposing an additional lysine binding site (LBS2) on free apo(a). This free apo(a) has an increased affinity to plasmin-modified fibrin, thereby impeding fibrinolysis and promoting atherothrombosis, consistent with the significant role apo(a) appears to play in transgenic mouse models of atherogenesis.39

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**Table 3. Relative Risk of CAD by Lp(a) and tHcy Levels by Sex**

<table>
<thead>
<tr>
<th>Lp(a), mg/dL</th>
<th>tHcy, $\mu$mol/L</th>
<th>Total Population</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>OR (95% CI; $P$)</td>
<td>n</td>
</tr>
<tr>
<td>&lt;30</td>
<td>&lt;17</td>
<td>588</td>
<td>1.0</td>
<td>402</td>
</tr>
<tr>
<td>&lt;30</td>
<td>$\geq$17</td>
<td>114</td>
<td>1.49 (0.90–2.45; 0.12)</td>
<td>82</td>
</tr>
<tr>
<td>$\geq$30</td>
<td>&lt;17</td>
<td>366</td>
<td>1.52 (1.09–2.12; 0.01)</td>
<td>213</td>
</tr>
<tr>
<td>$\geq$30</td>
<td>$\geq$17</td>
<td>85</td>
<td>2.72 (1.45–5.12; 0.002)</td>
<td>53</td>
</tr>
</tbody>
</table>

Test for interaction $P$ 0.60 0.56 0.03

Adjusted for age, race, smoking, diabetes, LDL-C, HDL-C, body mass index, systolic blood pressure, lipid-lowering medications, and current hormone replacement therapy in females.
between Lp(a) and tHcy in defining risk of CAD. Because this study was small and included only men <45 years of age, generalizability of these results may be limited.

Harpel et al. initially suggested that tHcy promoted binding of Lp(a) to plasmin-modified fibrin. This would potentially lead to more atherogenesis and atherothrombosis associated with elevations of both tHcy and Lp(a). It is now known that Lp(a) is composed of apo(a)-linked to an apoB-100-LDL particle by a single disulfide bond. Thiols, such as tHcy, are known to dissociate apo(a) from the Lp(a) complex, leading to the exposure of an additional lysine-binding site on apo(a). This additional lysine-binding site may increase the affinity of apo(a) for plasmin-modified fibrin, thus impeding fibrinolysis (Figure 3). This modification of Harpel’s original hypothesis explains how the presence of tHcy results in greater Lp(a) fibrin binding. This theory is consistent with the suggestion that apo(a) is the atherogenic moiety of Lp(a), as noted in transgenic mouse models.

There are several limitations of our analysis in this population. This study is a cross-sectional study of patients referred to a clinical operation, not a randomized trial, and as such, is limited by biases introduced to any analysis of this type. Our subjects represent a high-risk population referred to a tertiary care center with inherent selection, referral filter, and ascertainment biases. Significantly, CAD was determined on the basis of predefined clinical characteristics, and we were unable to assess quantitatively the severity of disease. Although no patient was specifically referred for an elevated tHcy or Lp(a) level, patients referred to the clinic are considered to be at higher-than-average risk for cardiovascular disease and its sequelae. tHcy is known to be elevated after an acute coronary event. While our values for tHcy were, on average measured at least 3 months after acute events, tHcy may remain elevated as a result of an acute coronary syndrome. All potential confounding variables could not be controlled for, and our results may be prone to survivor bias.

Furthermore, our subanalysis by age and sex relies on a relatively small number of women in the elevated tHcy and Lp(a) category (n=32). Wide CIs result, as reported in Table 3 (1.70 to 13.70). Therefore we caution against relying too heavily on the specific estimate of the risk associated with the joint presence of both risk factors. The risk estimated by the ORs does provide evidence to conclude that the combined risk in women exceeds that of simply combining (either additively or multiplicatively) the isolated risk of the respective risk factors. Finally, although no interaction was demonstrated in the total male population, this may represent survivor bias and may have been confounded by small sample size. There is evidence of a possible interaction in younger men. These results may reflect real sex and age differences in tHcy or Lp(a) thresholds for CAD risk, indicate a selection/referal bias, or suggest other unknown modifiers of these 2 CAD risk factors.

Our findings support the hypothesis that tHcy and Lp(a) interact to increase the risk of CAD. A high tHcy level may act in concert with a high Lp(a) level to promote atherosclerosis and or vascular disease. Although our clinic population represents a selective, high-risk group, these results provide evidence of important differences in the joint effect of Lp(a) and tHcy on CAD risk by sex and potentially, patient age, and add insights into the role of these 2 risk factors in the pathogenesis of atherosclerosis. These data provide an interesting hypothesis-generating finding regarding the differential interactive effects of 2 emerging cardiovascular risk factors and may have important implications for the prevention and treatment of CAD in select high-risk populations.

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