Gender Differences in Intermediate Atherogenic Pathways by Cigarette Smoking among Middle-Aged US Adults

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Abstract: *Objective*: This study investigates mediating atherogenic pathways caused by cigarette smoking (anthropometric, metabolic, hemodynamic, inflammatory factors) among middle-aged adults and whether there are gender differences in these pathways.

Methods: The data were obtained from the Los Angeles Atherosclerosis Study. The sample consists of 573 middle-aged healthy U.S. adults (age 40-60 yrs). Common carotid arterial intima-medial thickness (IMT) measured by B-mode ultrasound was used as a surrogate indicator for subclinical atherosclerosis.

Results and Conclusion: Besides high levels of low-density lipoprotein cholesterol and total cholesterol, cigarette smoking was also associated with manifestations of metabolic syndrome (central obesity, atherogenic dyslipidemia, sympathetic overactivity, elevated inflammation markers). Most intermediate physiologic profiles for former smokers were similar to those for never smokers, suggesting that smoking effects are partly reversible after quitting. The common atherogenic mediating pathways by smoking for men and women was central obesity. The unique pathway for women was dyslipidemia (low HDL cholesterol and high triglycerides), and the unique pathways for men were elevated levels of LDL cholesterol and total cholesterol, sympathetic overactivity, and elevated inflammation markers.

Keywords: Blood lipids, cholesterol, female, inflammation, insulin resistance, LDL, male, smoking.

INTRODUCTION

The association between cigarette smoking and cardiovascular morbidity and mortality has been well established [1]. The pathophysiology underlying cardiovascular risk caused by smoking is complicated. Smoking is adversely associated with endothelial dysfunction and arterial wall thickening [2, 3]. Smoking-accelerated atherosclerosis was recognized to be involved in the occurrence and progression of cardiovascular disease [4].

The atherogenic effects by cigarette smoking may be direct [5] or indirect by affecting a set of intermediate variables (i.e., mediators) that contribute to atherosclerosis. Regarding the mediating effects, besides elevated serum total cholesterol and low-density lipoprotein (LDL) cholesterol, cigarette smoking also increase the risk of metabolic syndrome [6, 7], which constitutes another important risk factor cluster for atherosclerosis [8].

The reports on the association between cigarette smoking and some manifestations of metabolic syndrome are inconsistent [9-13]. Furthermore, the relative difference on atherosclerosis and relevant risk factor profiles between current, former, and never smokers are not fully clear [14-16]. More evidence on the extent and on what aspects smoking cessation is related to reduced (or elevated) atherosclerotic risk is needed. In addition, it is controversial whether smoking causes atherosclerosis for men to a greater degree than for women [17, 18]. There are reports that some harmful effects of smoking on the arterial wall and atherogenic intermediate variables are gender-related [19-22]. However, the evaluation of the gender differences in the smoking-atherosclerosis association has rarely included the atherosclerosis markers and mediating variables simultaneously.

In the current study, common carotid arterial intima-medial thickness (CCA-IMT) measured by B-mode ultrasound was used as a surrogate indicator of subclinical atherosclerosis [23]. Increased carotid IMT has been shown to be directly associated with an increased risk of cardiovascular disease [24-26].

This study aimed to investigate: 1) mediating atherogenic pathways caused by cigarette smoking (anthropometric, metabolic, hemodynamic, inflammatory markers) among middle-aged adults, 2) whether adverse effects of smoking are reversible following smoking cessation, and 3) whether there are gender differences in these pathways.

METHODS

Study Cohort and Follow-Up

The initial Los Angeles Atherosclerosis Study (LAAS) cohort of 573 middle-aged adults (men, n=304, age 40-60 yr;

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Table 1. Baseline Demographic and Physiologic Characteristics of Study Participants by Gender. Los Angeles Atherosclerosis Study, 1995-1998

Variables	Men (n=304)	Women (n=269)	*p**
	No. (%) of Participants		
Ethnicity			.12
Hispanic	100 (32.9)	72 (26.8)	
Non-Hispanic white	166 (54.6)	149 (55.4)	
Black	13 (4.3)	18 (6.7)	
Asian	15 (4.9)	27 (10.0)	
Others	10 (3.3)	3 (1.1)	
Smoking status			.005
Current smokers	90 (29.6)	53 (19.7)	
Former smokers	90 (29.6)	69 (25.7)	
Never smokers	124 (40.8)	147 (54.7)	
Diabetes	9 (3.0)	7 (2.6)	.78
Antihypertensive medication	42 (13.8)	51 (19.0)	.12
Lipid-lowering medication	27 (8.9)	10 (3.7)	.01
Menopause	-	142 (61.0)	-
Use of oral contraceptives			-
Current users	-	7 (3.0)	
Former users	-	173 (74.6)	
Use of HRT			-
Current users	-	110 (47.4)	
Former users	-	22 (9.5)	
History of hysterectomy	-	82 (35.3)	-
	Mean (SD)		
Age, y	48.7 (4.7)	51.4 (4.4)	<.0001
Body height, m	1.76 (.07)	1.62 (.07)	<.0001
Mean IMT, μm	676 (105)	654 (88)	<.0001
Fasting insulin, log (pmol/L)†	4.69 (0.57)	4.58 (0.47)	.0057
Fasting glucose, log (mmol/L)†	1.74 (0.19)	1.68 (0.14)	.0001
Body mass index, kg/m^2	28.5 (4.8)	27.2 (5.9)	.002
Sagittal-transverse abdominal diameter ratio	0.681 (0.055)	0.642 (0.054)	<.0001
Fasting HDL-C, mmol/L	1.31 (0.24)	1.66 (0.37)	<.0001
Triglycerides, log (mmol/L)†	0.59 (0.55)	0.35 (0.56)	<.0001
Fasting LDL-C, mmol/L	3.68 (0.90)	3.24 (0.87)	<.0001
Fasting total serum cholesterol, mmol/L	5.65 (0.99)	5.50 (0.93)	.031
Systolic blood pressure, mmhg	129.6 (12.7)	127.7 (16.3)	.004
Diastolic blood pressure, mmhg	91.8 (8.9)	88.4 (9.8)	<.0001
C-reactive protein, log (mg/dL)†	0.294 (0.063)	0.680 (0.068)	<.0001

Note. IMT indicates intima-medial thickness; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. *Gender differences in continuous and categorical variables were determined by analysis of covariance (adjusted for age) and X^2 test of significance, respectively. † A logarithmically transformed mean and its standard deviation are shown.

women, n=269, age 45-60 yr) was recruited from random samples of a local utility company employees [27-29]. Hispanics and current smokers were oversampled. The participation rate was 85%. The exclusion criteria were: history of heart attack, angina, revascularization, stroke, or current cancer treatment. The baseline examination was completed in 1995. Two follow-up examinations were completed at 1.5-yr intervals. Out of the selected sample, 500 completed the 18-month (n=480), 36-month (n=447), or both follow-up examinations. The IMT progression was determined by the difference between baseline and 36-month (or 18-month if 36-month not available) follow-up after adjustment for the time between the two examinations. Fully informed consent was obtained from all the participants. The study protocol was approved by the Institutional Review Board of the Keck School of Medicine at the University of Southern California. The baseline characteristics of the study participants are listed in Table **1**. Approximately 47% of the participants were never smokers, with approximately equal proportions of current and former smokers (26%).

Predictor

Smoking status was defined as former, current, and never smokers. Former smokers were smokers who had already quit smoking before enrollment and remained abstinent during the follow-up.

Measurement of Potential Mediators

Fasting blood samples were collected by venipuncture and were frozen at -70^{o}C. Total serum cholesterol, high-density lipoprotein (HDL) cholesterol: triglycerides were measured by an autoanalyzer with the Roche direct HDL-cholesterol method which meets the 1998 NIH/NCEP goals for acceptable performance [30]. Low-density lipoprotein cholesterol (LDL-C) was estimated for fasting samples only (fasting time longer than 8 hours prior to examination) [31]. Plasma glucose concentration was measured by the glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin was determined by a specific radioimmunoassay with reagents from Linco Research (St. Louis, MO) with a detection limit of 2 microU/ml (12 pmol/l) and interassay coefficient of variation of 6-8%. High sensitivity C-reactive protein (Hs-CRP) was measured by Latex particle enhanced immunoturbidimetric assay (ITA) using an automated chemistry analyzer (Equal Diagnostics Company, Exton, PA).

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by standard sphygmomanometer and recorded to the nearest digit. Pulse pressure (PP) was the difference of SBP and DBP. Seated heart rate was assessed by measuring pulse at seated position. Two measurements were taken for SBP, DBP and heart rate and the average was used for each examination. Height, weight, sagittal abdominal diameter, and transverse abdominal diameter were measured. BMI was calculated as weight (kg) divided by height squared (m^2). The sagittal-transverse abdominal diameter ratio was obtained from the two diameters.

Outcome Measures

Common carotid artery intima-medial thickness (CCA-IMT) was assessed using high-resolution B-mode ultrasound (ATL scanner, model UM4+, with 7.5 MHz linear transducer). IMT was measured at the distal wall of the artery in the 1 cm section of the common carotid 0.25 proximal to the bulb. The subjects were scanned in two body positions (supine and lateral) and on both sides (right and left). Up to 8 frames were processed for each subject. Procedures for image acquisition and processing were reported previously [32]. The annual progression rate was calculated from the difference between baseline and 3-yr (or 1.5-yr if 3-yr measurement was missing) follow-up thickness reading after adjustment for the time between the two examinations.

Statistical Analysis

When considering the mediated (indirect) effects of cigarette smoking, we presume that some mediating variables (anthropometric, metabolic, hemodynamic, inflammatory markers) are more proximate in the atherogenic pathways than cigarette smoking. The effect of cigarette smoking (a distant variable) is passed on to atherosclerosis through those mediating variables, i.e., smoking-›mediators-›IMT. According to conventional definition, three criteria must be met to determine mediation [33]: (a) there must be a significant relation between smoking status and IMT (or IMT progression), (b) there must be a significant relation between smoking status and the mediating variable, (c) the mediator must be a significant predictor of IMT (or IMT progression) in an equation including both the mediator and smoking status.

Logarithmic transformation was performed on variables (fasting insulin, glucose, triglycerides, C-reactive protein) that were markedly skewed toward high values.

Least-square means (adjusted means) for IMT, IMT progression rate and potential mediators were obtained relative to smoking status groups after adjustment for age, ethnicity, diabetes status, use of antihypertensive and cholesterol-lowering medications. This analysis was carried out to examine the first two criteria of mediation, *i.e.*, a significant relation between smoking status and IMT (and IMT progression) and a significant relation between smoking status and mediating variables. A separate set of analysis was performed for women with further adjustment for menopausal status, oral contraceptive use, and use of hormone replacement therapy.

Partial correlation analysis was performed to further examine the third criteria of mediation, *i.e.*, whether a potential mediator is correlated to IMT and IMT progression after controlling for smoking status.

All analyses were performed in SAS 8.02 (SAS Institute Inc., Cary, NC). All reported p values are two-tailed with significance defined as $p<0.05$. Experimentwise error rate was set at .05 for Bonferroni correction when multiple groups were compared.

RESULTS

Current smoking was significantly related to greater carotid average IMT for men and more rapid IMT progression for women, compared with never smokers. For both men and women, current smoking was significantly associated with higher LDL cholesterol, total cholesterol, and central obesity (indicated by higher sagittal-transverse abdominal diameter ratio). Current smoking was significantly associated with elevated seated heart rate and C-reactive protein for men. In comparison, current smoking was significantly associated with higher triglycerides and lower HDL cholesterol for women (Table **2**).

Most cardiovascular risk factor levels were comparable between former smokers and never smokers. However, the carotid average IMT for former smokers was significantly greater than that for never smokers among men; former smoking was significantly associated with general obesity (indicated by BMI, compared with current and never smokers), central obesity (compared with never smokers); Cigarette smoking (current or former) was not significantly related to fasting plasma levels of insulin, glucose, or any blood pressure measures for both men and women.

The proposed potential mediators were all significantly correlated to either IMT or IMT progression in men and/or women after controlling for smoking status and other covariates (Table **3**).

Table 2. Summary of Potential Mediators and Outcomes by Smoking Status in Men and Women. Los Angeles Atherosclerosis Study, 1995-1998

	Never Smokers	Former Smokers	Current Smokers
Men (n=304)	n=124	n=90	n=90
Carotid average IMT, μm	**650.4.2±8.8**[ac]	**675.7±9.9**[ab]	**717.4±10.4**[bc]
IMT progression, μm/yr	9.0±1.8	9.9±2.1	9.6±2.2
Fasting insulin, log (pmol/L)*	4.651±0.052	4.785±0.059	4.641±0.062
HDL cholesterol, mmol/L	1.319±0.023	1.280±0.026	1.313±0.027
TG, log (mmol/L)*	0.550±0.052	0.618±0.058	0.598±0.061
Seated heart rate, beats/min	**76.3±1.1**[c]	**77.2±1.2**[b]	**82.6±1.3**[bc]
Hs-CRP, log (mg/dL)*	**0.010±0.095**[c]	**0.196±0.107**[b]	**0.621±0.112**[bc]
Women (n=269)	n=147	n=69	n=53
Carotid average IMT, μm	645.9±7.0	658.3±10.2	670.8±12.0
IMT progression, μm/yr	9.2±1.7	11.1±2.5	14.4±3.0
Fasting insulin, log (pmol/L)*	4.620±0.038	4.489±0.056	4.590±0.066
HDL-C, mmol/L	**1.693±0.030**[c]	**1.705±0.044**[b]	**1.483±0.054**[bc]
TG, log (mmol/L)*	**0.277±0.043**[c]	**0.304±0.063**[b]	**0.603±0.076**[bc]
Seated heart rate, beats/min	79.7±0.9	78.7±1.4	79.9±1.6
Hs-CRP, log (mg/dL)*	0.645±0.088	0.830±0.128	0.863±0.153
Men and Women (n=573)	n=271	n=159	n=143
Fasting glucose, log (mmol/L)*	1.705±0.009	1.711±0.012	1.705±0.013
BMI, kg/m^2	**27.58±0.31**[a]	**28.75±0.40**[ab]	**27.26±0.44**[b]
Rsag2Tr	**0.653±0.003**[ac]	**0.666±0.004**[a]	**0.672±0.005**[c]
LDL-C, mmol/L	**3.428±0.056**[c]	**3.330±0.072**[b]	**3.688±0.083**[bc]
Total cholesterol, mmol/L	**5.566±0.059**	**5.441±0.076**[b]	**5.753±0.084**[b]
SBP, mmhg	128.6±0.8	128.0±1.1	129.3±1.2
DBP, mmhg	90.0±0.6	89.7±0.7	90.7±0.8
Pulse pressure, mmhg	38.6±0.6	38.3±0.8	38.6±0.9

Note. Values are given as least-square mean±standard error. The least-square means (adjusted means) were computed using GLM in SAS with adjustment for age, ethnicity, diabetic status, and use of antihypertensive and cholesterol-lowering medications. The analysis was performed for women and men separately if the smoking by sex interaction was detected. The variables with the same superscriptions across groups are significantly different with experimentwise error rate at .05 according to modified Bonferroni method.
Rate of change in IMT was determined by three examinations. All other variables were taken from baseline examination. IMT, intima-medial thickness; BMI, body mass index; Rsag2Tr, sagittal/ transverse abdominal diameter ratio; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hs-CRP, high-sensitivity C-reactive protein. * A logarithmically transformed mean and its standard error are shown.

Based on the three criteria of mediation, the common atherogenic mediating pathways by smoking for men and women was central obesity; The unique pathway for women was dyslipidemia (low HDL cholesterol and high triglycerides), and the unique pathways for men were elevated levels of LDL cholesterol, total cholesterol, sympathetic overactivity (indicated by elevated seated heart rate), and inflammation (indicated by C-reactive protein).

For women, a separate analysis was performed with further adjustment for menopausal status, oral contraceptive use, and use of hormone replacement therapy. No dramatic differences were observed in comparison with the aforementioned results.

DISCUSSION

The main finding of this study was that smoking was associated with a series of risk factors that mediated early atherosclerosis. Some of the mediating pathways were common across gender, and others were gender-specific.

The lack of association between habitual cigarette smoking and fasting insulin may not necessarily indicate that smoking is not related to insulin resistance [11]. First, hyperinsulinemia may not be an ideal marker for insulin resistance [34]. Second, the epidemiological evidence linking smoking with insulin resistance is considerable. Studies using more accurate measures on insulin resistance (e.g., euglycemic clamp technique, frequently sampled intravenous glucose tolerance test) concluded that smoking is indeed related to insulin resistance [7, 35]. Third, smoking may interfere with beta-cell function. Cigarette smoking men had a lower homeostasis model assessment (HOMA) value than ex-smokers and never smokers [36]. There was evidence that current smoking was associated with lower fasting insulin compared with that in nonsmokers [13]. The

present study also indicated that current smoking was associated with lower fasting insulin compared with that in former smokers. Thus, the simultaneous effects of damaged insulin secretion and increased insulin resistance caused by smoking may diminish the differences of fasting insulin levels between smokers and non-smokers and may even reverse the relations. This may be the underlying cause of the dissociation between smoking and hyperinsulinemia [13].

Table 3. Partial Correlation Coefficients Between IMT, IMT Progression and Potential Mediators. Los Angeles Atherosclerosis Study, 1995-1998

	IMT		IMT Progression	
	Women	**Men**	**Women**	**Men**
Fasting insulin	0.228***	0.189***	0.049	0.020
Fasting glucose	0.131+	0.030	-0.069	-0.007
BMI	0.409***	0.202**	0.073	0.127*
RSag2Tr	0.288***	0.158*	0.003	-0.053
HDL-C	-0.215**	-0.093	0.013	-0.141*
TG	0.130+	0.128*	0.133+	0.032
LDL-C	-0.024	0.216**	0.035	0.042
Total cholesterol	-0.056	0.160*	0.088	0.064
SBP	0.338**	0.294***	0.100	0.055
DBP	0.076	0.311***	-0.068	0.092
Pulse pressure	0.388***	0.107+	0.194**	-0.013
Seated heart rate	-0.042	0.061	-0.078	0.144*
Hs-CRP	0.176**	0.141*	0.129+	-0.096

Note. The Pearson partial correlation coefficients were obtained with adjustment for smoking status, age, body height, ethnicity, diabetic status, and use of antihypertensive and cholesterol-lowering medications. $+p<0.10$ $*p<0.05$ $**p<0.01$ $***p<0.001$. IMT, intima-medial thickness; BMI, body mass index; Rsag2Tr, sagittal/ transverse abdominal diameter ratio; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hs-CRP, high-sensitivity C-reactive protein.

Elevated sympathetic nervous system activity is a characteristic trait of the metabolic syndrome [37, 38] and a risk factor for atherosclerosis [39]. An acute effect of cigarette smoking is to increase the activity of the sympathetic nervous system and the levels of circulating catecholamines [40, 41]. In habitual smokers, smoking one cigarette increases heart rate, blood pressure and cardiac output [42]. The current study showed no evidence of habitual smoking as a risk factor for hypertension, which is consistent with some of other studies [22]. However, current smoking was indeed associated with elevated habitual heart rate (for men only), which is in agreement with other studies [42].

The gender differences on the atherogenic pathways caused by cigarette smoking have important etiologic and therapeutic implications. Habitual smoking was more related to dyslipidemia in middle-aged women compared with men, whereas smoking was more related to sympathetic overactivity and elevated C-reactive protein in middle-aged men compared with women. Based on the current study, blood lipid levels (especially HDL cholesterol and triglycerides) should be routinely examined for women habitual smokers for the cardiovascular risk reduction efforts. Male habitual smokers should be more aware of the acute atherothrombotic events (such as myocardial infarction, stroke) because elevated C-reactive protein suggests active inflammation predictive of an early cardiovascular outcome [43]. However, there are also reports that smoking is related to dyslipidaemia for men [44, 45] and smoking is related to elevated C-reactive protein for women as well [46]. In a study among patients with chronic coronary artery disease, cigarette smoking is associated with increased circulating proinflammatory and procoagulant markers, which are associated with endothelial dysfunction, and atherosclerosis [47, 48]. Further epidemiological studies with larger smoker samples are warranted to confirm our findings. In addition, the short follow-up period (3-year) limited our ability to some extent for detecting significant associations between risk factors and IMT progression.

Former smokers had a carotid IMT that was in-between that of never smokers and current smokers among men. On one hand, part of the adverse smoking effects is irreversible [3]. On the other hand, some atherogenic risk factors might rise among former smokers. For example, former smokers were in general more obese [49]. In our study, current smokers had lowest BMI among all three groups (especially among men), which is consistent with other reports [13]. Less general obesity in smokers may offer them some protection against progression of atherosclerosis [11]. There was also evidence that more quitters become hypertensive than non-quitters, although the groups had similar baseline blood pressure levels [50]. Weight gain subsequent to cessation probably contributed to this excess incidence of hypertension in quitters [50]. However, in the current study, we only identified the group differences in the extent of obesity but not in the levels of blood pressure.

In contrast with men, no significant difference on carotid IMT was observed for women across the three smoking status groups. However, habitual smoking was indeed associated with dyslipidemia (high triglycerides, low HDL cholesterol) in women, which is a significant atherogenic factor. How could this paradox be interpreted? Another study using the same cohort [27] revealed that cigarette smoking was related to carotid intimal thickening (which is supposed to increase IMT) and medial atrophy (which is supposed to decrease IMT) in women, which resulted in the dissociation between smoking status and overall carotid intima-medial thickness. In contrast, there was no significant smoking effect on medial thickness for men; the change in overall IMT thus reflected change in intimal thickness. Therefore, smoking is atherogenic among women as well and may even cause more harm than that among men. IMT *per se* may not be a valid indicator for smoking-induced atherosclerosis among women.

When both smoking status and significant mediating variables were taken into account simultaneously, smoking status (current and former smoking) was still significantly associated with the baseline IMT for men (data not shown), supporting that both direct and indirect effects were involved in smoking-induced atherosclerosis and that cigarette smoking is an independent atherogenic factor.

CONCLUSIONS

Cigarette smoking contributed to atherosclerosis by gender-differential intermediate pathways. Some of the pathways are metabolic syndrome-associated. The effects of cigarette smoking on atherosclerosis and intermediate risk factors may be partially reversible after quitting. The findings from this study can be used to educate adult men and women to quit smoking in order to reduce overall cardiovascular risk.

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