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# Risk Factors Associated With Increased Carotid Intima-Media Thickness in a Male Population With Chronic Alcohol Consumption

## *A Prospective Observational Study*

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**Abstract:** Previous studies have reported a relationship between alcohol consumption and carotid intima-media thickness (CIMT). However, the exact associations between different severities of CIMT and dyslipidemia, dyslipoproteinemia, inflammatory immune markers, and oxidative markers associated with chronic alcohol consumption remain unknown.

The aim of this study was to explore whether there are associations between different severities of CIMT and dyslipidemia, dyslipoproteinemia, inflammatory immune markers, and oxidative markers associated with chronic alcohol consumption.

We enrolled 173 males with chronic alcohol consumption and categorized them into 2 groups: 104 chronic alcohol consumers with normal CIMT (group A) and 69 chronic alcohol consumers with increased CIMT (group B).

Nonparametric statistics showed that age, body mass index (BMI), and serum TC, TG, Apo A1, and ApoB levels were significantly higher in group B than in group A ( $P=0.002, 0.019, 0.021, 0.023, 0.001,$  and  $0.001$ , respectively). Additionally, tumor necrosis factor alpha (TNF $\alpha$ ) and HSP70 serum levels were significantly lower in group B than in group A ( $P=0.023$  and  $0.017$ , respectively). A binary logistic regression analysis showed that age (OR: 1.077, 95% CI: 1.024–1.13,  $P=0.004$ ), ApoB (OR: 6.828, 95% CI: 1.506–30.956,  $P=0.013$ ), and TNF- $\alpha$  (OR: 0.999, 95% CI: 0.998–1.00) were independent risk factors associated with CIMT.

The present study demonstrated that age, ApoB, and TNF $\alpha$  are independent risk factors associated with CIMT. Thus, older subjects with increased serum ApoB levels are more likely to present with increased CIMT, suggesting that age and ApoB promote such thickening and that TNF $\alpha$  downregulation might play a protective role against

the progression of subclinical atherosclerosis in subjects with chronic alcohol consumption.

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**Abbreviations:** ApoA1 = apolipoprotein A1, ApoB = apolipoprotein B, BMI = body mass index, CIMT = carotid intima-media thickness, HSP70 = heat shock protein 70, LDL = low-density lipoprotein, MDA = malondialdehyde, SOD = superoxide dismutase, sOX40L = soluble OX40 ligand, TC = total cholesterol, TG = triglyceride, TNF $\alpha$  = tumor necrosis factor alpha.

## INTRODUCTION

Previous research has revealed that carotid intima-media thickness (CIMT) is significantly associated with future cardiovascular events and that CIMT can be reliably determined by carotid ultrasound.<sup>1</sup> Therefore, CIMT is commonly used as an accessible and reliable measure for assessing subclinical atherosclerosis. In addition, several studies have shown that dyslipidemia, dyslipoproteinemia, the inflammatory immune response, and lipid peroxidation are associated with atherosclerosis. However, previous studies have reported conflicting results regarding the correlation between alcohol consumption and atherosclerosis. For example, one study showed that binge drinking correlated with increased atherosclerotic progression in middle-aged males; however, this increase was not associated with total alcohol consumption.<sup>2</sup> Other studies have indicated a positive correlation between alcohol consumption and CIMT in healthy males aged 30 to 70 years<sup>3</sup> and in young adults.<sup>4</sup> Contradictory conclusions were reported in studies showing that alcohol consumption is inversely correlated with CIMT,<sup>5,6</sup> inversely related to CIMT in males but not in females<sup>7</sup> and positively correlated with carotid plaques in males but not in females.<sup>6</sup> However, few studies have explored the associations between different severities of CIMT and blood lipids, lipoproteins, inflammatory immune markers, and oxidative markers associated with chronic alcohol consumption. To address this knowledge gap, we investigated these relationships.

## CLINICAL DATA

### Ethics Statement

The study protocol was approved by the ethics committee of Taishan Hospital of Shandong Province. All subjects provided written informed consent prior to participation.

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## Study Population

Based on ultrasound investigation, 173 inpatient males with chronic alcohol consumption who underwent a general health examination were recruited from January 2012 to May 2015. The subjects were grouped based on the presence or absence of increased CIMT; CIMT less than 0.09 cm was considered normal, and CIMT greater than or equal to 0.09 cm was considered abnormally increased.<sup>8</sup> Chronic alcohol ingestion was determined based on daily ethanol intake >40 g for a period >5 years.<sup>9</sup> All of the participants were divided into 2 groups according to the CIMT ultrasonography results: 104 chronic alcohol consumers with normal CIMT (group A) and 69 chronic alcohol consumers with increased CIMT (group B). The aim of this study was to investigate the correlations between CIMT and dyslipidemia, dyslipoproteinemia, inflammatory immune markers, and oxidative balance by detecting serum lipids (total cholesterol [TC] and triglyceride [TG]), serum lipoproteins (apolipoprotein A1 [ApoA1] and apolipoprotein B [ApoB]), tumor necrosis factor alpha (TNF- $\alpha$ ), E-selectin, soluble OX40 ligand (sOX40L), heat shock protein 70 (HSP70), malondialdehyde (MDA), and superoxide dismutase (SOD). Subjects that met at least one of the following criteria were excluded from the study: age <25 years or >65 years, smoking, infection, pregnancy, various types of virus hepatitis, cirrhotic patients, primary and/or secondary heart, head, endocrine, nervous, or hematological diseases, or mental disorders.

## Experimental Apparatus and Reagents

Experimental apparatus utilized for this study included a color ultrasound system (GEV7 and LOG7, GE, USA), standard plate reader (ANTHOS2010, Austria), and automatic biochemical analyzer (7080, Japan). In addition, the reagents used for this study included enzyme-linked immunosorbent assay kits for TNF $\alpha$ , E-selectin, sOX40L, HSP70, MDA, and SOD (provided by Shanghai Enzyme-Linked Immune Co., Ltd.; all kits manufactured by R&D Co., USA).

## CIMT Measurements

To measure CIMT, all subjects were instructed to lie in the supine position, and the carotid ultrasound scans were performed by an ultrasonologist using a high-resolution B-mode ultrasound with a 7.5 MHz linear-array transducer.<sup>3</sup> The average CIMT was calculated using our previous measurement method.<sup>8</sup>

## Laboratory Measurements

Venous blood samples taken from above the elbow from each subject in the morning after a 10 hours overnight fast were centrifuged at 3000 rpm for 10 minutes, and serum was then collected and frozen at  $-70^{\circ}\text{C}$  until analysis. Blood lipids and lipoproteins were measured using an automatic biochemistry analyzer. Serum levels of TNF $\alpha$ , E-selectin, sOX40L, HSP70, MDA, and SOD were detected by enzyme-linked immunosorbent assay. All the assays were conducted in accordance with the manufacturer's instructions.

## Statistical Analysis

All analyses were performed with IBM SPSS Statistics 19 (IBM, Inc., Armonk, NY). All continuous data are expressed as the median and range. The comparisons between groups were performed using the Mann-Whitney *U*-test. Then, logistic regression models were used to estimate the multivariate odds ratios (ORs) and 95% confidence intervals (CIs) for the independent risk factors associated with CIMT. Statistical significance was defined by a 2-sided  $P \leq 0.05$ .

## RESULTS

The differences in age, body mass index (BMI), blood lipids, apolipoproteins, inflammatory cytokines, and lipid peroxidative markers between the 2 groups are presented in Table 1. The subjects in group B were significantly older and had a significantly higher BMI than those in group A

**TABLE 1.** Comparisons of Age, BMI, Blood Lipid and Apolipoprotein, Inflammatory Cytokine, and Lipid Peroxidative Marker Between the 2 Groups (Medians Plus Ranges)

Group	A	B	Z	P
N	104	69		
Age, year	44.00 (25.00–62.00)*	55.00 (33.00–65.00)	3.118	0.002
BMI, kg/m <sup>2</sup>	25.75 (19.50–35.90)*	26.90 (19.50–33.50)	2.350	0.019
A mean alcohol drinking history, years	5.00 (5.00–13.00)	5.00 (5.00–14.00)	1.187	0.069
A mean daily alcohol consumption, g	56.00 (40.00–99.00)	56.00 (41.00–99.00)	0.025	0.980
Blood lipid and apolipoprotein				
TC, mmol/L	5.39 (3.03–7.35)	5.62 (3.92–11.42)	2.313	0.021
TG, mmol/L	1.47 (0.37–9.11)	1.81 (0.59–10.11)	2.666	0.023
ApoA1, mmol/L	1.30 (0.74–2.07)	1.52 (0.83–2.36)	3.302	0.001
ApoB, mmol/L	1.05 (0.46–1.88)	1.17 (0.57–1.67)	3.341	0.001
Inflammatory cytokine				
TNF- $\alpha$ , ng/L	373.40 (76.00–2078.00)	212.20 (1.00–1337.00)	2.274	0.023
sOX40L, ng/L	11.55 (0.40–80.00)	8.16 (1.11–66.50)	1.421	0.155
HSP70, ng/L	65.85 (9.10–385.50)	44.70 (22.30–311.00)	2.384	0.017
E-selectin, ng/L	44.75 (4.00–265.00)	32.60 (13.00–165.00)	1.248	0.212
Oxidative balance marker				
MDA, ng/L	6.45 (1.00–43.00)	5.27 (2.00–41.00)	1.725	0.084
SOD, U/mL	66.95 (10.00–329.00)	67.70 (5.00–311.00)	0.650	0.516

ApoA1 = apolipoprotein A-I, ApoB = apolipoprotein B, BMI = body mass index, HSP70 = heat shock protein 70, MDA = malondialdehyde, SOD = superoxide dismutase, sOX40L = soluble OX40 ligand, TC = total cholesterol, TG = triglyceride, TNF- $\alpha$  = tumor necrosis factor- $\alpha$ .

( $Z = 3.118$ ,  $P = 0.002$  and  $Z = 2.350$ ,  $P = 0.019$ , respectively). The mean alcohol drinking history and mean daily alcohol consumption did not significantly differ between groups B and A ( $Z = 1.187$ ,  $P = 0.069$  and  $Z = 0.025$ ,  $P = 0.980$ , respectively). The serum levels of TC, TG, ApoA1, and ApoB were significantly higher in group B than in group A ( $Z = 2.313$ ,  $P = 0.021$ ;  $Z = 2.666$ ,  $P = 0.023$ ;  $Z = 3.302$ ,  $P = 0.001$ ; and  $Z = 3.341$ ,  $P = 0.001$ , respectively).

A comparison of inflammatory cytokine serum levels between the 2 groups showed that TNF $\alpha$  and HSP70 serum levels were significantly lower in group B than in group A ( $Z = 2.274$ ,  $P = 0.023$  and  $Z = 2.384$ ,  $P = 0.017$ , respectively). However, serum sOX40L and E-selectin levels did not significantly differ between the 2 groups ( $Z = 1.421$ ,  $P = 0.155$  and  $Z = 1.248$ ,  $P = 0.212$ , respectively). Additionally, the serum levels of the oxidative balance markers MDA and SOD did not significantly differ between the 2 groups ( $Z = 1.725$ ,  $P = 0.084$  and  $Z = 0.650$ ,  $P = 0.516$ , respectively).

A binary logistic regression analysis (Table 2) showed that age (OR: 1.077, 95% CI: 1.024–1.13,  $P = 0.004$ ), ApoB (OR: 6.828, 95% CI: 1.506–30.956,  $P = 0.013$ ), and TNF- $\alpha$  (OR: 0.999, 95% CI: 0.998–1.00) were independent risk factors associated with CIMT.

## DISCUSSION

There are numerous factors associated with atherosclerosis, especially age and BMI, which are important risk factors for cardiovascular and cerebrovascular diseases. A previous study revealed that the CIMT-related factors included sex, age, orthostatic blood pressure change, and lack of alcohol intake in community-dwelling elderly subjects aged 80 years or older.<sup>10</sup> In addition, age may be an important influential factor in the relationship between alcohol consumption and atherosclerotic risk factors, and a significant association between alcohol intake and a lower risk for metabolic syndrome has been demonstrated in young, but not in elderly, males.<sup>11</sup> Our previous study showed that CIMT correlated with age and metabolic factors in subjects with alcoholic fatty liver disease.<sup>12</sup> Additionally, previous studies have shown that the CIMT quartile is significantly higher in subjects with a higher BMI.<sup>13,14</sup> The present study found that among patients who ingested alcohol, those with increased CIMT were older than were those with a normal CIMT. Furthermore, the BMI was higher in the alcohol-ingesting group with increased CIMT than in that without an increased CIMT, suggesting an association between BMI, CIMT, and alcohol consumption. However, the binary logistic regression analysis revealed that only age is an independent risk factor associated with increased CIMT.

Large-scale studies have shown that blood lipids and lipoproteins are associated with atherosclerosis and atherosclerosis-related diseases. Therefore, the associations between

CIMT, an early atherosclerosis marker, and blood lipids and lipoproteins are of particular interest in the clinic. Logistic regression analysis revealed that the concentrations of TC, low-density lipoprotein (LDL)-cholesterol, and TG were significantly associated with elevated CIMT after adjusting for age and sex.<sup>15</sup> A previous study demonstrated an association between CIMT and LDL-cholesterol but not high-density lipoprotein cholesterol (HDL-C) or TG.<sup>16</sup> Another study showed that postprandial triglyceridemia might be an independent risk factor for early atherosclerosis, suggesting that it is a better predictor of CIMT than particle concentrations of individual TG-rich lipoproteins.<sup>17</sup> Additionally, serum ApoB and ApoA1 levels can be estimated by measuring the serum levels of TC, HDL-C, and TG, and the apolipoprotein estimates could be routinely added to laboratory reports to complement lipoprotein lipids for risk assessment.<sup>18</sup> A recent study demonstrated that CIMT in healthy children at 5 years is associated with their apolipoprotein profile.<sup>19</sup> ApoA-I-containing HDL-mimetic particles may reverse atherogenic changes in the arterial wall in conjunction with maximal LDL-lowering therapy.<sup>20</sup> Serum ApoB levels<sup>15</sup> and the ApoB/ApoA-I ratio<sup>15,21</sup> have shown significant associations with CIMT; thus, serum ApoB levels and the ApoB/ApoA-I ratio could potentially be used as independent predictors of subclinical atherosclerosis. Moreover, serum ligand-containing ApoB was related to higher CIMT in Caucasian men in the US independent of other risk factors.<sup>22</sup> An opposing report stated that CIMT was not associated with ApoA isoforms.<sup>23</sup> Furthermore, plasma ApoB levels were not an independent predictive indicator associated with ultrasonographic phenotypes of carotid atherosclerosis in patients with diabetes mellitus.<sup>24</sup> Our study showed that serum TC, TG, ApoA1, and ApoB levels were higher in alcohol-consuming subjects with increased CIMT; hence, increased CIMT was associated with age, BMI, TC, TG, ApoA1, and ApoB in subjects who consume alcohol. However, the binary logistic regression analysis revealed that ApoB, but not TC, TG, or ApoA1, is an independent risk factor for increased CIMT. Nevertheless, the serum lipid constituents that promote (i.e., TC, TG, and ApoB) or reduce (i.e., ApoA1) atherosclerosis are all involved in alcohol-related thickening of CIMT. Taken together, these results indicate that only ApoB is an independent risk factor associated with increased CIMT and alcohol consumption. The association between alcohol consumption and increased CIMT remained unclear because there are many confounding factors, such as the role of alcohol itself, varieties and quantities of drinking, and food composition parameters. The manner in which these factors contribute to the observed differences should be clarified in the future.

Inflammatory immune responses are also a leading cause of atherosclerosis. However, the roles of inflammatory immune responses in carotid atherosclerosis remain unknown. Alcohol alters cytokine levels in plasma and in various tissues, including the lung, liver, and brain.<sup>25</sup> In moderation, alcohol may lower inflammatory marker levels and, accordingly, the risk of cardiovascular disease (CVD).<sup>26</sup> Lowering low-grade inflammation and endothelial activation may be potential mechanisms by which alcoholic beverages exert their cardioprotective effects.<sup>27</sup> Plasma TNF $\alpha$  and interferon- $\gamma$  levels were significantly higher in an ethanol-treatment group compared to a control group.<sup>28</sup> Alcohol consumption might alter serum TNF $\alpha$  and IL-6 levels<sup>29</sup> and result in dysregulated cytokine levels (IL-10, IL-12, and interferon- $\gamma$ ).<sup>30</sup> Alcohol-induced endothelial damage or protection may also be associated with the synthesis or activation of certain markers, such as nitric

**TABLE 2.** Binary Logistic Regression Analyses Results

Variables	Coefficient	SE	Wald	OR (95% CI)	P Value
Age	0.074	0.026	8.335	1.077 (1.024–1.132)	0.004
ApoB	1.921	0.771	6.206	6.828 (1.506–30.956)	0.013
TNF- $\alpha$	-0.001	0.001	3.931	0.999 (0.998–1.000)	0.047

ApoB = apolipoprotein B, CI = confidence interval, OR = odds ratio, SE = standard error, TNF- $\alpha$  = tumor necrosis factor- $\alpha$ .

oxide, endothelin-1, adhesion molecules, TNF $\alpha$ , IL-6, and CRP.<sup>31</sup> Moderate drinkers may have lower serum levels of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin than heavy drinkers.<sup>32</sup> Furthermore, E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 were upregulated in liver biopsies from patients with alcoholic hepatitis and cirrhosis.<sup>33</sup> HSP70 and sOX40L might play a dual role in atherosclerosis. A randomized study<sup>34</sup> demonstrated that extracellular HSP70 was significantly lower in a population with atherosclerosis and that both HSP70 and sOX40L might be markers of the progression of atherosclerotic disease. However, other studies have drawn the opposite conclusion, that is, increases in CIMT at follow-up were less prevalent in subjects with high serum HSP70 levels,<sup>35</sup> which correlated with different severities of atherosclerosis in patients with different severities of carotid artery disease and chronic lower limb ischemia.<sup>36</sup> Serum sOX40L levels independently correlate with CIMT, suggesting a potential relationship between sOX40L and atherosclerosis.<sup>37</sup> In addition, elevated serum sOX40L levels indicate an increased risk for cardiovascular events in subjects with ACS.<sup>38,39</sup> Moreover, sOX40L may serve as a potential marker for predicting the severity of coronary heart diseases.<sup>39</sup> The results of the present study indicated that the inflammatory markers TNF $\alpha$  and HSP70 were downregulated in alcohol-consuming subjects with increased CIMT, leading to the hypothesis that alcohol may lower certain inflammatory markers. Moreover, the binary logistic regression analysis revealed that decreased serum TNF $\alpha$  levels might be a protective factor associated with increased CIMT and alcohol consumption. Thus, the role of alcohol-related inflammatory immune responses in alcohol-consuming subjects with increased CIMT remains unknown, and further investigations are required.

Previous studies have suggested that chronic alcohol ingestion may change oxidative stress and cytokine induction, which are associated with free radicals.<sup>28</sup> Chronic alcoholics have markedly increased erythrocyte lipid MDA concentrations and SOD activity.<sup>40</sup> Another study confirmed that alcohol consumption resulted in significantly elevated levels of lipid peroxidation products, such as MDA, hydroperoxides, and conjugated dienes, in the liver. Furthermore, SOD, catalase, glutathione reductase, and glutathione contents were decreased in the liver.<sup>41</sup> Oxygen free radicals are involved in the pathogenesis of atherosclerosis. A nested case-controlled study showed that CIMT was associated with markers of oxidative damage to lipids and DNA, independent of conventional risk factors for CVD.<sup>42</sup> The present study revealed that the levels of MDA, a lipid peroxidation product, and SOD activity were not significantly associated with increased CIMT in subjects who consumed alcohol. Therefore, the precise mechanisms responsible for this association require further study.

In conclusion, the data suggest that increased CIMT is associated with advanced age; higher BMI, TC, TG, ApoA1, and ApoB values; and TNF $\alpha$  and HSP70 downregulation in subjects with chronic alcohol consumption. However, the binary logistic regression analysis demonstrated that age, ApoB, and TNF $\alpha$  are independent risk factors associated with CIMT. Thus, older subjects with increased serum APOB levels are more likely to present with increased CIMT, suggesting that age and ApoB promote such thickening and that TNF $\alpha$  downregulation might play a protective role against the progression of subclinical atherosclerosis in subjects with chronic alcohol consumption. This potential discrepancy requires further research.

Our study has particular limitations. First, certain influential factors for atherosclerosis, such as sex and smoking history as well as the use of lipid-lowering medication, were not considered. Second, this study did not differentiate the effects of the variety or quantity of alcohol consumed on CIMT. Third, our conclusions are based on a cross-sectional study rather than a randomized controlled study. Fourth, there was an insufficient number of research subjects included in this study; accordingly, studies with a larger cohort are needed to confirm the hypotheses suggested herein.

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