Intima-Media Thickness of Lower-Limb Arteries Associated with Fasting and Post-Challenge Plasma Glucose Levels

Hung-Chi Ho\textsuperscript{1,2}, Ming-Fong Chen\textsuperscript{1}, Juey-Jen Hwang\textsuperscript{1,3}, Yuan-Teh Lee\textsuperscript{1,2}, and Ta-Chen Su\textsuperscript{1}

\textsuperscript{1}Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan
\textsuperscript{2}Division of Cardiology, Department of Medicine, China Medical University Hospital, Taichung, Taiwan
\textsuperscript{3}Department of Internal Medicine and Cardiovascular Center, Yu-Lin Branch of National Taiwan University Hospital, Douliu, Yu-Lin, Taiwan

\textbf{Aim:} Atherosclerosis is a systemic disease with focal cardiovascular events. Although the accelerated development of peripheral arterial disease in diabetic patients is well known, the pathogenic mechanism of site-specific susceptibility to glycemia is uncertain.

\textbf{Objective:} To investigate the association of fasting and post-challenge glucose levels with intima-media thickness (IMT) at different arterial sites.

\textbf{Methods:} Forty consecutive middle-aged volunteers aged 37 to 53 years were recruited to define the association of IMT with cardiovascular risk factors at 12 carotid and 6 lower-limb arterial sites. A linear mixed model was used to regress the primary outcome measures, which were repeated measures of IMT at multiple arterial sites (18 sites per participant), on fixed-effect predictors of various conventional cardiovascular risk factors, while accounting for the interdependence of repeated measures taken from the same participant with unstructured covariance.

\textbf{Results:} Carotid IMTs were associated independently with waist circumference and systolic blood pressure, whereas lower-limb IMTs were associated with waist circumference, low-density lipoprotein cholesterol (LDL-C), glycated hemoglobin A1C (HbA1C), and fasting and 2-hour post-challenge plasma glucose levels; these associations were stronger in overall arteries. Independent associations of ALT and smoking with IMT appeared only in overall arteries.

\textbf{Conclusion:} In a middle-aged, nonclinical sample, lower-limb but not carotid IMTs are associated independently with HbA1C, and fasting and 2-hour post-challenge plasma glucose levels.

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\textbf{Key words:} Site-specific atherosclerosis, Linear mixed model, Oral glucose tolerance test, Intima-media thickness

\textbf{Introduction}

Carotid IMT, a surrogate marker for atherosclerosis, is linked to the prediction of future cardiovascular events in epidemiologic studies\textsuperscript{1} and the progress and regression of atherosclerotic disease in intervention trials\textsuperscript{2}. Since atherosclerosis is an insidious disease that starts early in life, the identification and management of modifiable determinants of IMT in young, asymptomatic individuals may have a significant impact on preventive medicine. Ignoring site-specific predisposition to various risk factors and restricting IMT measurement to a limited number of arterial sites may underestimate the importance of a risk factor. Instead, considering risk factors in terms of their site-specific effect on IMT provides a better insight into the mechanism of atherosclerosis and lends more support to risk stratification. Diabetics are known to be at increased risk for peripheral arterial disease\textsuperscript{3}; however, there are limited data that addressing the propensity of lower-limb arteries, as compared
with other parts of the body, to develop atherosclerosis.

This is a study on the association of conventional cardiovascular risk factors with IMT measured at 12 carotid and 6 lower-limb arterial sites in middle-aged participants. Specifically, the study aimed to investigate the association of fasting and post-challenge plasma glucose levels with IMT at different arterial sites.

**Methods**

**Subjects**

Forty consecutive middle-aged volunteers were recruited for a cardiovascular health examination at the National Taiwan University Hospital (NTUH) from 2001 through 2002. For reliability analysis, the first 20 participants returned 2 weeks later for repeated measurement of IMT at 18 arterial sites. All participants provided written informed consent. The research ethics committee of the NTUH approved this study.

**Demographic and Laboratory Data**

Systolic and diastolic blood pressures (BP) were recorded using a mercury sphygmomanometer in a standardized procedure and are reported as the mean of 2 replicate measurements taken 5 minutes apart with the participant resting in a seated position. Body mass index (BMI) was computed as body weight in kilograms divided by the square of the body height in meters. Information about tobacco smoking and alcohol habits was obtained as part of a self-reported questionnaire.

A standard 75-gram oral glucose tolerance test (OGTT) was performed on all participants after an overnight fast of at least 10 hours, with measurements of fasting, 1-hour and 2-hour post-challenge plasma glucose from blood samples drawn via an ante-cubital vein. Plasma glucose and serum levels of total cholesterol (TC), triglycerides (TG), LDL-C, high-density lipoprotein cholesterol (HDL-C), and alanine aminotransferase (ALT) were also measured using an autoanalyzer (Hitachi 7250 Special; Hitachi, Tokyo, Japan).

**Ultrasonographic IMT Measurement**

High-resolution, real-time B-mode vascular ultrasound for carotid and lower-limb arteries were performed using a Hewlett-Packard SONO 4500 ultrasound system (Andover, MA) with a linear array 3- to 11-MHz transducer. The same experienced ultrasonographer who was blinded to the medical status of each participant performed all scans and image measurements. Ultrasound images were recorded on super-VHS videotapes for subsequent off-line analysis.

Arterial far-wall IMT was measured bilaterally, on longitudinal scans, at 0–1 cm (CCA1) and 1–2 cm (CCA2) proximal to the carotid bulbs, in the proximal (BULB1) and distal (BULB2) half of the bulb, in the internal (ICA) and external (ECA) carotid artery within 1 cm distal to the flow divider of the bulb, within 1 cm before the femoral (CFA) and popliteal (POPA) bifurcation, and in the proximal 1 cm of the posterior tibial artery (POTA). The carotid bulb was defined as the segment between the point where the common carotid artery lost its parallel wall and the tip of the carotid flow divider. IMT was defined as the distance between the two echogenic lines identified on B-mode images which represent the leading edges of the lumen-intima and media-adventitia interfaces, respectively.

**Statistical Analysis**

Demographic features, baseline clinical and laboratory values, and IMT at each arterial site were compared between the reliability group and all study participants using either *t*-test for the means of two independent samples or the Chi-square test for proportions. Continuous variables were expressed as the mean ± sample standard deviation.

All available IMT determinants were analyzed using a linear multivariate mixed model. In this situation, repeated IMT measurements at various arterial sites of a given participant (18 sites per participant) were regressed simultaneously on the fixed-effect factors of conventional IMT determinants while accounting for the interdependence of repeated measures taken from the same participant with an unstructured covariance. This covariance structure was determined among several other commonly used structures using two methods: a graphical depiction of sample covariances as a function of lag in arterial sites and using information criteria such as Akaike information criteria (AIC). In the unstructured covariance structure, all conventional IMT determinants were assumed to have fixed effects in order to estimate covariance parameters. Restricted maximum likelihood (REML) procedures were used to estimate and assess the significance of the coefficients of conventional IMT determinants and covariance parameters. In this model, the 18 arterial sites were treated as a random sample from the population of all possible arterial sites in the body and the statistical inference of interest was meant for the population rather than for any specific site per se.

Mixed model diagnostics were performed using residual and influence analyses. In residual analysis, the marginal studentized residuals were distributed
approximately normally on the normal plot and had a mean of nearly zero; there were no conspicuous outliers. In influence analysis, Cook’s D statistics for the fixed effects were distributed evenly across participants, indicating no influential observations.

To investigate the site-specific, differential strength of the association of IMT with its determinants, the 18 arterial sites were subdivided into the 12-site carotid group (bilateral CCA1, CCA2, BULB1, BULB2, ICA, and ECA) and the 6-site lower-limb group (bilateral CFA, POPA, and POTA), to which the above statistical procedures were applied separately.

To evaluate the intra-class correlation coefficient of reliability (ICCR) of IMT measurements, 20 of the 40 participants were recruited for repeat IMT measurement at each of the 18 arterial sites 2 weeks later. The ICCR was defined as the ratio of between-participant variance to total variance under the framework of one-way random effect analysis of variance. An ICCR of <0.4 was considered poor, 0.4–0.75 good, and >0.75 excellent. All statistical analyses were performed using SAS (Release 9.1.3; SAS Institute Inc., Cary, NC, USA). SAS PROC MIXED was used to carry out linear mixed model analysis.

**Results**

The baseline characteristics and OGTT results of the 40 study participants are shown in Table 1. The average of age, BMI, and SBP was 45.7 years, 23.8 kg/m², and 120.5 mmHg, respectively. The first 20 study participants enrolled for IMT ICCR evaluation showed no significant difference from all study participants in all characteristics.

The IMT measurements were generally reliable and reproducible, with an ICCR of 0.64 for all 18 arterial sites taken as a whole (Table 2). The carotid IMTs were associated with systolic and diastolic BPs,
while the IMTs of lower-limb arteries were associated with the ratio of LDL-C to HDL-C, HbA1C, and post-challenge plasma glucose levels (Table 3). These associations tended to be stronger for the IMTs of the total arteries.

Multivariate linear mixed modeling revealed that
the association of plasma glucose levels, especially HbA1C, with the IMTs of lower-limb (but not carotid) arteries remained independently significant after adjusting for age, sex, waist circumference, systolic BP, ALT, serum lipids, and smoking and alcohol habits (Table 4). LDL-C was an additional independent determinant of IMT exclusively at lower-limb arteries, while systolic BP was exclusively at carotid arteries. Associations of the above risk factors with IMT tended to be stronger in overall arteries. Waist circumference was consistently an independent determinant across groups of arterial sites and different plasma glucose indexes. The independent associations of ALT and smoking with IMT appeared only in overall arteries.

Table 4. Multivariate $\beta$ coefficients of various IMT determinants by arterial sites and plasma glucose indexes, using linear mixed models for repeated measures

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Carotid$^1$</th>
<th>Lower-limb$^2$</th>
<th>Total$^3$</th>
<th>Carotid</th>
<th>Lower-limb</th>
<th>Total</th>
<th>Carotid</th>
<th>Lower-limb</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.134 **</td>
<td>-0.2056</td>
<td>-0.1275</td>
<td>-0.0790</td>
<td>-0.0733</td>
<td>-0.3097**</td>
<td>0.0015</td>
<td>0.0031</td>
<td>-0.1441</td>
</tr>
<tr>
<td>Male</td>
<td>0.0035</td>
<td>-0.0025</td>
<td>0.0124</td>
<td>0.0066</td>
<td>0.0061</td>
<td>0.0068</td>
<td>0.0057</td>
<td>-0.0065</td>
<td>0.0038</td>
</tr>
<tr>
<td>Age, 102 y</td>
<td>-0.2750</td>
<td>0.0792</td>
<td>0.0642</td>
<td>-0.3543</td>
<td>-0.0844</td>
<td>0.1936</td>
<td>-0.3345</td>
<td>0.0565</td>
<td>0.1127</td>
</tr>
<tr>
<td>Waist, m</td>
<td>0.4715 **</td>
<td>0.3696 **</td>
<td>0.2616 **</td>
<td>0.4206 **</td>
<td>0.3336 **</td>
<td>0.3801 **</td>
<td>0.4366 **</td>
<td>0.3921 **</td>
<td>0.4422 **</td>
</tr>
<tr>
<td>SBP, 102 mmHg</td>
<td>0.2530 **</td>
<td>0.0500</td>
<td>0.0663</td>
<td>0.2375 **</td>
<td>0.0267</td>
<td>0.0603</td>
<td>0.2424 **</td>
<td>0.0328</td>
<td>0.0675</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>0.0008</td>
<td>0.0003</td>
<td>0.0015 **</td>
<td>0.0007</td>
<td>0.0003</td>
<td>0.0015 **</td>
<td>0.0008</td>
<td>0.0001</td>
<td>0.0014 **</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>0.0186</td>
<td>0.0234 **</td>
<td>0.0165 **</td>
<td>0.0147</td>
<td>0.0277 **</td>
<td>0.0238 **</td>
<td>0.0166</td>
<td>0.0250</td>
<td>0.0216 **</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>-0.0091</td>
<td>-0.0006</td>
<td>-0.0078</td>
<td>-0.0058</td>
<td>-0.0016</td>
<td>-0.0135 **</td>
<td>-0.0075</td>
<td>0.0006</td>
<td>-0.0093</td>
</tr>
<tr>
<td>Smoking drinking</td>
<td>-0.0110</td>
<td>0.0432</td>
<td>0.0286 **</td>
<td>-0.0148</td>
<td>0.0345</td>
<td>0.0311 **</td>
<td>-0.0110</td>
<td>0.0353</td>
<td>0.0228</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>-0.0141</td>
<td>-0.0148</td>
<td>-0.0136</td>
<td>-0.0110</td>
<td>-0.0245</td>
<td>-0.0218</td>
<td>-0.0132</td>
<td>-0.0128</td>
<td>-0.0077</td>
</tr>
<tr>
<td>HbA1C, %</td>
<td>0.0099</td>
<td>0.0424 **</td>
<td>0.0389 **</td>
<td>0.0097</td>
<td>0.0255</td>
<td>0.0369 ***</td>
<td>-0.0012</td>
<td>0.0050</td>
<td>0.0068 *</td>
</tr>
</tbody>
</table>

Values are estimates of $\beta$ coefficients.

1 Includes bilateral CCA1, CCA2, BULB1, BULB2, ICA, and ECA
2 Includes bilateral CFA, POPA, and POTA
3 Includes bilateral CCA1, CCA2, BULB1, BULB2, ICA, ECA, CFA, POPA, and POTA

ALT, alanine aminotransferase; AUC, area under curve; HbA1C, glycated hemoglobin A1C; LDL-C, low-density-lipoprotein cholesterol; PC1h, 1-hour post-challenge plasma glucose; PC2h, 2-hour post-challenge plasma glucose; SBP, systolic blood pressure; TG, triglycerides.

Discussion

The present study extends previous findings that IMTs at different carotid arterial sites (CCA, BULB, and ICA) have different strengths of association with systolic BP, hypertension, and diabetes mellitus. Moreover, cardiovascular risk factors have different impacts on IMT at carotid and lower-limb arteries. Carotid IMTs are strongly associated with waist circumference and systolic BP. In lower-limb arteries, IMTs are associated independently with waist circumference, LDL-C, and post-challenge plasma glucose levels. The site-specific variability of the association of IMT with different cardiovascular risk factors is also evidenced by previous studies on its regression after statin treatment.

To the best of our knowledge, the present study is the first to show that the relationship between glycemia, specifically HbA1C, fasting plasma glucose, and 2-hour post-challenge plasma glucose, and IMT is statistically significant at lower-limb arteries but not carotid arteries. This is in line with a population-based survey analysis showing a progressive relation between categories of impaired glucose regulation and IMT, with the strength of association greater at femoral than carotid arteries, upon adjusting for the conventional cardiovascular risk factors of waist circumference, LDL-C, HDL-C, systolic BP, and smoking.
the gradual accumulation of advanced glycation end (AGE) products results in the accelerated development of peripheral arterial disease in diabetic patients, but the pathogenic mechanism of site-specific susceptibility to glycemia remains uncertain in humans. This question was partly answered by Litwak et al. who randomly assigned 16 cynomolgus monkeys to an experimentally induced diabetes group or control group, with 8 monkeys in each group. After 6 months of study, they concluded that, more significantly at femoral arteries than at carotid arteries, chronic hyperglycemia increases arterial LDL accumulation and atherosclerosis extent and also impairs arterial remodeling, which was defined in terms of the arterial luminal area to intimal area relationship. The increased LDL accumulation was thought to be mediated by AGE products, which enhanced the retention and diminished the removal of LDL in the arterial wall. The role of post-challenge hyperglycemic spike in the development of diabetic complications has also been demonstrated previously.

The significant and independent association of ALT with IMT observed in the analysis of all arteries, but not in carotid or lower-limb arteries alone, extends the results of a number of earlier studies on the prospective effect of ALT on coronary heart disease (CHD) and all-cause mortality to sub-clinical atherosclerosis. The strong association of ALT with carotid IMT has been well-established in previous studies.

A graded, positive association of ALT with CHD has been reported in 1,439 Caucasian subjects aged 50–75 years and in 247 female Iranian candidates for coronary angiography. Nakamura and colleagues have shown a positive relation of ALT and all-cause mortality in 4,524 Japanese men and women with BMI below the median (22.7 kg/m²). A graded, positive association of ALT with CHD has been reported in 1,439 Caucasian subjects aged 50–75 years and in 247 female Iranian candidates for coronary angiography. Nakamura and colleagues have shown a positive relation of ALT and all-cause mortality in 4,524 Japanese men and women with BMI below the median (22.7 kg/m²). The present study is one of the few to use linear mixed models for the analysis of repeated measures IMT. Our extensive measurement of IMT at 18 arterial sites for each participant has not been performed previously. In contrast to other studies using standard multiple regression analysis with an aggregate number (e.g., maximum IMT or the mean of several IMTs) as the outcome variable, we used linear mixed models, adopting a special covariance structure to account for the interdependence of repeated measures taken from the same participant. Linear mixed modeling for repeated measures encompasses modeling between-subject effects (fixed and/or random) and within-subject covariance patterns. Modeling of the covariance pattern is a major hurdle in linear mixed modeling and affects the inclusion of a random between-subject effect. In longitudinal data analysis, when repeated measures are taken over time, a commonly used linear mixed model includes a first-order auto-regressive covariance structure and a random, between-subject time effect; however, when the repeated measures are taken over space, as in this study of IMT being measured at 18 arterial sites of each participant, an "unstructured" covariance pattern is favored over any mathematically constrained pattern since the correlation between repeated measures cannot be accounted for by a single variable, such as time or distance. From a mathematical point of view, the selection of an unstructured covariance pattern obviates a random between-subject effect but has the disadvantage of having a large number of parameters to be estimated. The advantages of linear mixed modeling over traditional methods of repeated-measures analysis have been discussed in detail elsewhere and are shown in Table 5, taking HbA1C and total arterial area as an example. Since fixed effects, such as HbA1C, in the mixed model accounted for the mean value of repeated measures at the 18 arterial sites, in a sense very much like a predictor in common multivariate regression analysis that treated the mean IMT as the outcome variable, we obtained comparable β coefficients for the two models (0.03893 vs. 0.02676); however, the standard error for the mixed model was much less than that for the common regression analysis (0.009159 vs. 0.02198), producing a much more significant p value. This comparison approach has been used in Omar et al.

In conclusion, the plasma glucose indexes, specifically HbA1C, and fasting and 2-hour post-challenge plasma glucose levels, are independently associ-

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>β (SE) of HbA1C</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum IMT</td>
<td>0.1280 (0.06385)</td>
<td>0.0545</td>
</tr>
<tr>
<td>Mean IMT</td>
<td>0.02676 (0.02198)</td>
<td>0.2332</td>
</tr>
<tr>
<td>Repeated measures IMT (linear mixed model)</td>
<td>0.03893 (0.009159)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

HbA1C, glycated hemoglobin A1C; IMT, intima-media thickness; SD, standard error
ated with lower-limb but not carotid IMTs in a middle-aged, nonclinical sample. Such findings indicate that site-specific susceptibility of IMT to different cardiovascular risk factors should be taken into account in clinical decision-making.

Acknowledgement and Notice

There are no conflicts of interest to be disclosed.

References

5) Su TC, Jeng JS, Chien KL, Sung FC, Hsu HC, Lee YT: Hypertension status is the major determinant of carotid atherosclerosis: a community-based study in Taiwan. Stroke, 2001; 32: 2265-2271
10) Su TC, Lee YT, Chou S, Hwang WT, Chen CF, Wang JD: Twenty-four-hour ambulatory blood pressure and duration of hypertension as major determinants for intima-media thickness and atherosclerosis of carotid arteries. Atherosclerosis, 2006; 184: 151-156
24) Cheng KS, Mikhailidis DP, Hamilton G, Seifalian AM: A review of the carotid and femoral intima-media thickness as an indicator of the presence of peripheral vascular dis-
31) Sookoian S, Pirola CJ: Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: A systematic review. J Hepatol, 2008; [Epub ahead of print]