

# Skin cholesterol content identifies increased carotid intima-media thickness in asymptomatic adults

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**Background** A noninvasive assay to measure skin Tc recently has become available for use in the outpatient setting as a cardiovascular (CV) risk prediction tool. The purpose of this study was to determine whether skin tissue cholesterol content (skin Tc) levels are associated with increased carotid intima-media thickness (CIMT) after adjusting for known CV risk factors and Framingham CV risk.

**Methods** Consecutive patients without known vascular disease who were referred for determination of CIMT underwent B-mode ultrasonography of the carotid arteries and measurement of skin Tc using a noninvasive assay. Use of medications, cardiac risk factors, and Framingham 10-year CV risk were determined prospectively. Multivariable regression was used to determine predictors of increased CIMT.

**Results** Among 81 subjects, the mean (SD) age was 55.6 (7.7) years and the mean skin Tc was 95.9 (18.3) U. Carotid intima-media thickness was significantly higher among individuals in the highest quartile of skin Tc (0.87 vs 0.76 mm,  $P = .011$ ). In multivariable analyses, skin Tc was associated with increased CIMT even after adjusting for age, sex, glucose, systolic blood pressure, total/high-density lipoprotein cholesterol ratio, and use of lipid-lowering therapy (odds ratio [OR] per 10-unit increase = 1.590, 95% CI 1.525-1.658,  $P = .031$ ). Skin Tc also was associated with increased CIMT after adjustment for Framingham risk (OR = 1.341, 95% CI 1.302-1.380,  $P = .048$ ).

**Conclusions** Skin Tc is an easy-to-measure, noninvasive marker that can help identify subclinical atherosclerosis in asymptomatic middle-aged adults, even after controlling for risk factors and CV risk predicted by the Framingham model. (*Am Heart J* 2005;150:1135-9.)

Histological studies have demonstrated that skin tissue cholesterol content (skin Tc) is associated with aortic and coronary artery sterol and lipid deposition.<sup>1</sup> Biochemical assays have further shown that cholesterol quantified in the outermost layer of the epidermis, the stratum corneum, correlates well with epidermal cholesterol content.<sup>2</sup> An assay that measures cholesterol content within the stratum corneum and does not require a skin biopsy is available for outpatient measurement of skin Tc and potentially for cardiovascular (CV) risk prediction.<sup>2</sup> Using this assay, increased skin Tc has been associated with positive exercise treadmill stress tests and both the presence and extent of angiographic coronary artery disease (CAD) in individuals with anginal symptoms who were referred

for stress testing and/or coronary angiography.<sup>2,3</sup> A univariable association between skin Tc and Framingham CV risk also has been reported. The ability of skin Tc to predict CV disease in asymptomatic individuals in addition to standard CV risk factors, however, is not known.<sup>4</sup> Furthermore, the relationship between skin Tc and subclinical atherosclerosis has not been studied previously.

Carotid intima-media thickness (CIMT) is a validated measure of subclinical atherosclerosis that identifies both prevalent and incident CV disease in middle-aged and older adults.<sup>5-10</sup> The purpose of this study was to determine whether skin Tc levels are associated with increased CIMT even after adjusting for known CV risk factors and CV risk using the Framingham risk prediction model.

## Methods

### Experimental protocol

The Institutional Review Board of the University of Wisconsin Medical School approved this study. Data were obtained from consecutive, asymptomatic patients without manifest CV disease who were referred by their physicians to the University of Wisconsin Vascular Health Screening Program for CIMT measurement from March through October of 2003. The basis

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**Table 1.** Characteristics of study population (N = 81)

Age, y	55.6 (7.7)
Females, %	35.8
Mean skin tissue cholesterol content, U	95.9 (18.3)
Left-hand	94.7 (22.4)
Right-hand	97.1 (19.5)
Composite mean carotid-intima media thickness, mm	0.795 (0.141)
Lipids, mg/dL	
Total cholesterol	201.1 (42.5)
Triglycerides	134.9 (85.6)
High-density lipoprotein cholesterol	57.9 (16.8)
Low-density lipoprotein cholesterol	116.0 (37.3)
Total/high-density lipoprotein cholesterol ratio	3.6 (0.9)
Use of lipid-lowering therapy, %	39.5
Glucose, mg/dL	96.9 (15.1)
Systolic blood pressure, mm Hg	132.3 (14.0)
Use of antihypertensive therapy, %	14.8
Framingham 10-y cardiovascular risk, %	6.6 (5.9)

All values are mean (SD).

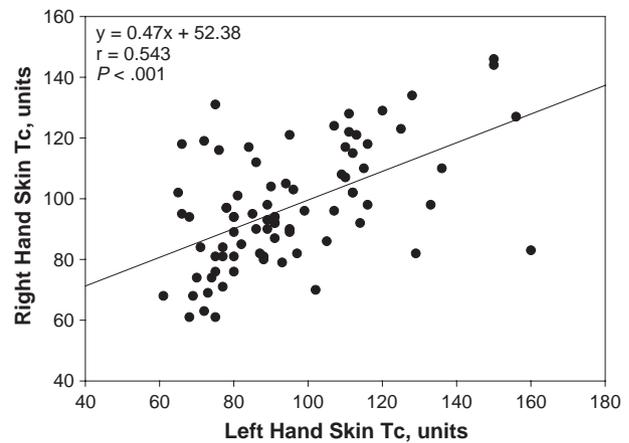
for referral was a physician's determination of "intermediate" CV risk in each subject. Subject characteristics such as age, sex, presence of CV risk factors, use of medications, and Framingham 10-year predicted risk of myocardial infarction or coronary death were determined prospectively.<sup>11</sup>

Blood samples were obtained after patients had fasted for at least 12 hours. Fasting plasma glucose, serum total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using standard enzymatic assays in a clinical laboratory whose procedures have been verified by the College of American Pathologists and Pacific Biometrics Proficiency Testing. Blood pressure was measured in each arm and averaged.

### Measurement of skin Tc

Skin Tc was determined noninvasively using the Cholesterol 1,2,3 system (International Medical Innovations, Toronto, Ontario, Canada).<sup>2-4</sup> The hypothenar eminence of each hand was cleansed with alcohol and allowed to dry. In a die-cut well foam template affixed to the palm, a drop of solution containing a synthetic digitonin-copolymer-horseradish peroxidase conjugate was applied to the prepared area and incubated for 1 minute. The area was blotted and an indicator solution containing a horseradish peroxidase substrate that induces a blue color change was applied in the well. Positive and negative control well samples were applied concurrently on the same skin surface. Skin cholesterol levels were quantified based on color change. Hue development was measured by reflectance using a handheld spectrophotometer (MD22 Spectrophotometer; X-Rite, Inc, Grandville, MI), which was interfaced with a computer, and the resulting change in hue was numerically reported in units (U). Assay validity was assessed by visual interpretation of control wells.

Previous studies have demonstrated that this hue measurement exhibits sufficient scope and dose-dependence to serve as a quantitative measure of skin Tc.<sup>2</sup> The manufacturer-reported reference range for asymptomatic adults >18 years old is 61-134 U (median, 93 U).<sup>2</sup> Within-day repeated measurements have demonstrated coefficients of variation of 5% to 19%

**Figure 1**

Skin Tc = skin tissue cholesterol content

Correlation between right- and left-hand skin Tc.

(mean, 11%) in subjects with mean skin Tc of 74 to 109 U, and interassay assessments on differing days have shown coefficients of variation of 2% to 12% (mean, 7%; mean skin cholesterol of 86-111 U).<sup>2</sup> No significant race or sex differences in skin Tc measured by this method have been observed. The statistical analyses and results reported below are based on the average of right- and left-hand skin Tc measurements.

### Carotid ultrasound imaging

The standardized imaging protocol from the Atherosclerosis Risk in Communities study was used to acquire images of the far walls of each carotid artery.<sup>12</sup> The common carotid artery segment was defined as the distal 1 cm immediately proximal to the onset of increased spatial separation of the walls of the common carotid artery (ie, before the origin of the bulb). The carotid bifurcation segment was defined as the distal 1 cm of the bulb, the termination of which was characterized by the presence of the flow divider between the internal and external carotid artery. The internal carotid artery segment was defined as the proximal 1 cm of the internal carotid artery, starting immediately beyond the flow divider. Ultrasound images were acquired using an 8.0-MHz linear array transducer (Acuson Sequoia, Siemens Medical Solutions, Malvern, PA) and recorded digitally using a Camtronics Vericis acquisition module (Camtronics Medical Systems, Hartland, WI). This module converts analogue video output from the ultrasound system into a proprietary digital image format for reading and measurement. Because no videotape is involved, image degradation is minimal, and resolution is limited by the ultrasound system and transducer, not the format or manipulation of the images. In this laboratory, image resolution is 85.67 pixels per cm (0.11 mm per pixel). The mean combined thickness of the intimal and medial layers of the far walls of each carotid artery segment was measured using proprietary software (CIMT Screen, Camtronics Medical Systems).<sup>2,3</sup> Composite CIMT was calcu-

**Table II.** Univariable correlates of skin tissue cholesterol content

	r	P
Age	0.129	.252
Carotid intima-media thickness	0.245	.028
Lipids		
Total cholesterol	0.017	.884
Triglycerides	0.091	.432
High-density lipoprotein cholesterol	-0.031	.788
Low-density lipoprotein cholesterol	-0.020	.863
Glucose	0.263	.022
Use of lipid-lowering therapy	0.065	.563
Systolic blood pressure	0.358	.001
Framingham 10-y cardiovascular risk	0.158	.171

lated as the mean CIMT of all measured carotid segments. On duplicate scanning, the reproducibility of composite CIMT values in this laboratory is  $0.004 \pm 0.087$  mm.<sup>13</sup> The reading software has been externally validated by an independent, blinded reference ultrasound laboratory (Center for Medical Ultrasound CIMT Reading Center, Wake Forest University School of Medicine, Winston-Salem, NC). The interlaboratory correlation coefficient for composite CIMT measurements is 0.98 ( $P < .0001$ ) (unpublished data).

### Statistical analysis

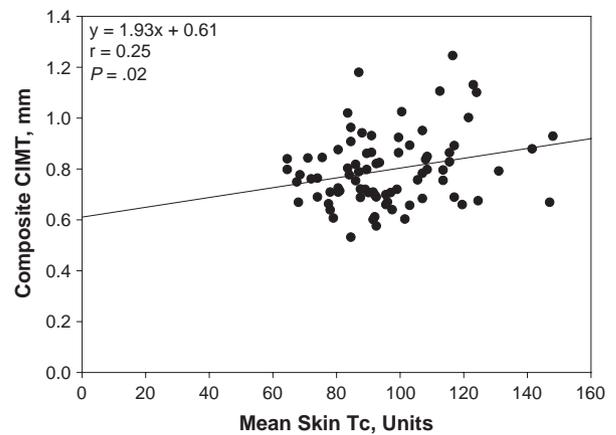
Statistical analyses were performed using the SAS system (version 8.2, SAS Institute, Chicago, IL) and SigmaStat (version 3.0, SPSS Inc, Chicago, IL). Means and standard deviations were computed and are reported for descriptive variables. Paired *t* tests were used to compare mean right- and left-hand skin Tc and CIMT values. Paired *t* tests were also performed to compare composite CIMT in the 75th versus the  $\leq 25$ th percentiles (highest and lowest quartiles, respectively) of mean skin Tc. Univariable (Pearson) correlations were determined between right- and left-hand skin Tc values and between subject characteristics, CV risk factor levels, Framingham CV risk, composite CIMT, and mean skin Tc values. Multivariable regression was used to determine predictors of mean CIMT in the highest quartile of the study population, incorporating the following independent variables: skin Tc, age, glucose, male sex, use of lipid-lowering therapy, systolic blood pressure, and total cholesterol/HDL-C ratio. A separate multivariable analysis was conducted with the 75th percentile composite CIMT as the dependent variable and both Framingham CV risk and mean skin Tc as independent variables. Odds ratios (OR) per 10-unit increase in skin Tc with 95% CIs were determined from these models. All analyses were performed with  $\alpha = .05$ .

## Results

### Subject characteristics

Characteristics of the 81 subjects enrolled are described in Table I. The mean (SD) age was 55.6 (7.7) years. Only 1 subject smoked cigarettes and only 1 had diabetes mellitus. Mean skin Tc was 95.9 (18.3) U (range, 64.5-148.0 U). The mean of differences between the right- and left-hand skin Tc values was 2.4 (20.2) U,

**Figure 2**



CIMT = carotid intima-media thickness  
Skin Tc = skin tissue cholesterol content

Correlation between mean skin Tc and composite CIMT.

which were not significantly different ( $P = .293$ ; coefficient of variation, 21.8%). The correlation between skin Tc values from each hand was strong ( $r = 0.543$ ,  $P < .001$ ) (Figure 1). The average Framingham 10-year predicted risk of myocardial infarction or coronary death was 6.6 (5.9)%. The mean composite CIMT was 0.795 (0.141) mm. This value approximately represents the 63rd percentile for age, sex, and race-adjusted CIMT in the Atherosclerosis Risk in Communities Study.<sup>14</sup> Composite CIMT was significantly higher among individuals in the highest quartile (0.874 [0.166] mm) than in the lowest quartile of skin Tc (0.760 [0.095] mm) ( $P = .011$ ). In subjects not on lipid-lowering therapy ( $n = 49$ ), CIMT still was significantly higher in the highest vs lowest quartiles of skin Tc (0.856 [0.166] mm vs 0.747 [0.100] mm,  $P = .050$ ).

### Correlates of skin Tc

In univariable analyses (Table II), significant correlations were observed between skin Tc and fasting serum glucose ( $r = 0.263$ ,  $P = .022$ ), systolic blood pressure ( $r = 0.358$ ,  $P = .001$ ), and mean CIMT ( $r = 0.245$ ,  $P = .028$ ) (Figure 2). Among those not on lipid-lowering therapy, a significant correlation between skin Tc and mean CIMT still was present ( $r = 0.308$ ,  $P = .031$ ). None of the lipid levels correlated significantly with skin Tc, nor did Framingham risk score (Table II).

### Skin Tc as a predictor of increased CIMT

Average skin Tc was a strong predictor of CIMT in the highest quartile (OR per 10-unit increase in skin

**Table III.** Multivariable predictors of increased mean carotid intima-media thickness

	OR	95% CI	P
Male sex	54.368	4.845-610.133	.001
Use of lipid-lowering therapy	6.206	1.270-30.329	.024
Skin Tc, per 10 units	1.590	1.525-1.658	.031
Age, y	1.092	0.977-1.221	.119
Systolic blood pressure, mm Hg	1.062	0.994-1.134	.074
Glucose, mg/dL	0.964	0.917-1.013	.147
Total/high-density lipoprotein cholesterol ratio	0.673	0.265-1.709	.405

OR odds ratio.

Tc = 1.413, 95% CI 1.373-1.454,  $P = .017$ ), even after adjusting for age, male sex, glucose, systolic blood pressure, total cholesterol/HDL-C ratio, and use of lipid-lowering therapy (OR = 1.590, 95% CI 1.525-1.658,  $P = .031$ ) (Table III). In a separate analysis, skin Tc was still associated with increased CIMT even after adjusting for predicted CV risk using the Framingham model (OR = 1.341, 95% CI 1.302-1.380,  $P = .048$ ).

## Discussion

In this study, increased skin Tc independently predicted increased CIMT among asymptomatic middle-aged adults. Specifically, a 10-unit increase in skin Tc was associated with an approximately 1.5-fold increase in the odds of having CIMT in the highest quartile, indicating the presence of increased subclinical atherosclerosis and increased CV risk. This relationship remained statistically significant even after controlling for traditional CV risk factors and predicted CV risk using the Framingham model. These findings suggest that skin Tc may help identify subclinical atherosclerosis in asymptomatic middle-aged adults, even in the presence of traditional risk factors. Skin Tc is easy to measure and may be a useful office-based tool for CV risk prediction.

This is the first study to show an independent association between skin Tc and subclinical atherosclerosis using this validated, surrogate marker of CV risk. Skin Tc previously has been associated with the presence and extent of angiographic CAD in middle-aged adults referred for cardiac catheterization because of angina and a positive stress test.<sup>3</sup> In that population of symptomatic individuals, Framingham CV risk was associated with increased skin Tc.<sup>3</sup> In another study of individuals with angina, increased skin Tc was associated with having a positive treadmill stress test.<sup>2</sup> Skin Tc also predicted increased Framingham predicted CV risk in asymptomatic, middle-aged individuals; however, that study included some subjects with known atherosclerotic disease and only a univariable relation between skin Tc and CV risk was reported.<sup>4</sup> A significant

univariable association between skin Tc and intercellular adhesion molecule-1 also was observed, with trends for significant correlations with C-reactive protein and vascular adhesion molecule-1.<sup>4</sup> These findings lend support to our observation that skin Tc is associated with increased CIMT in this middle-aged study group.

Skin Tc also has been shown to be lower in healthy subjects than individuals with CAD. In 115 healthy subjects who were age- and sex-matched with individuals undergoing coronary angiography, skin Tc levels in the healthy subjects were lower than in all subjects, including those with no angiographic CAD.<sup>2,15</sup> In another study of 60 subjects (55 without vascular disease), individuals without vascular disease and with low Framingham CV risk had lower skin Tc than subjects with vascular disease or higher risk scores.<sup>4</sup> Prospective, longitudinal studies to evaluate the relationship between skin Tc and several markers of vascular disease are in progress, so it is not clear whether or not increased skin Tc levels precede the development of atherosclerosis.

In addition to skin Tc, other significant predictors of increased CIMT in our study were male sex and concurrent lipid-lowering therapy. Indeed, male sex was a powerful predictor of increased CIMT in the multivariable model; however, creating separate models for each sex was not possible because of the relatively small number of individuals with CIMT in the highest quartiles (13 men, 7 women). In general, however, the relationships between skin Tc and CIMT were similar among men and women.

## Limitations

Nearly half of the study group was on lipid-lowering therapy; however, the relationship between skin Tc and CIMT remained significant in the subjects not on lipid-lowering therapy. The multivariable analyses could not be meaningfully replicated in this group because of the insufficient power of the smaller sample; however, a significant, independent relationship between skin Tc and angiographically documented CAD has been demonstrated previously in symptomatic subjects not on lipid-lowering therapy.<sup>3</sup> The prevalent use of lipid-lowering therapy in this study may have mitigated the associations between skin Tc, serum lipid levels, and Framingham CV risk; however, the relationships we identified were, in general, similar to those reported previously in the literature.<sup>2-4</sup> The correlation between skin Tc and CIMT may similarly have been confounded.

In this study, skin Tc did not correlate with serum lipids. The lack of association between serum lipids and skin Tc is interesting and is a consistent finding in all of the previous studies looking at skin Tc levels. For example, the largest study found no correlations between skin Tc and serum total cholesterol, LDL-C, and HDL-C levels; however, a modest association with triglycerides ( $r = 0.10$ ,  $P = .02$ ) was observed among 649

subjects with predominantly symptomatic CAD.<sup>3</sup> Two other studies showed univariable associations between total cholesterol, LDL-C, and skin Tc, but these relationships did not remain significant in multivariable analysis.<sup>2-4</sup> It is not clear why serum cholesterol levels are not strongly related to skin Tc. Skin Tc may relate more directly to the extent or duration of dyslipidemia. The presence of cholesterol analogs in the skin, such as plant sterols, could also confound an association with serum cholesterol. Plant sterols in particular can accumulate in the skin and bind with high affinity to the assay's digitonin conjugates; however, epidermal concentrations of plant sterols are low, even in subjects with high dietary consumption.<sup>2</sup> As discussed above, prevalent use of lipid-lowering therapy may also have obscured the relationship between cholesterol values and skin Tc, as lipid-lowering therapy may differentially affect serum and skin cholesterol concentrations. Finally, the sample size may have been too small to detect a significant association. It is noteworthy, however, that despite the modest correlation between skin Tc and CIMT, skin Tc still independently predicted atherosclerosis in this middle-aged population. Whether skin Tc measurements can predict incident CV events is not known and should be further investigated in a larger study; however, its associations with increased CIMT, a well-validated marker of subclinical CV disease and future CV risk, provide support for skin Tc as a potentially useful clinical risk assessment tool.

Because of the very low prevalence of diabetes mellitus and smoking in this cohort, these characteristics were not included as covariates in the first multivariable model (Table III); however, we adjusted for fasting glucose levels. Smoking status was included in the second model, as a component of the Framingham risk score. Also, although we did not adjust for hypertension history in the first model, systolic blood pressure levels were included, and in the second model use of antihypertensive therapy and systolic blood pressure were included as components of the Framingham risk score. Despite the adjustments, skin Tc significantly predicted elevated CIMT in both models.

## Conclusions

Skin Tc is a noninvasive marker that can help identify subclinical atherosclerosis in asymptomatic middle-aged adults, even after controlling for risk factors and CVD risk predicted by the Framingham model. Because skin Tc is easy to measure, it may be a useful office-based tool for cardiovascular risk prediction.

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