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Association of Skin Cholesterol Content, Measured by a Noninvasive Method, With Markers of Inflammation and Framingham Risk Prediction

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A novel, noninvasive test has been developed that provides a quantitative assessment of cholesterol in the skin.[1] Zawydiwski et al[1] reported a relation between the magnitude of the skin cholesterol test result and the presence or absence of an abnormal treadmill stress test. The association between the skin cholesterol and the treadmill results were not solely or even closely related to the ambient, serum cholesterol values, suggesting that the skin test may be affected by other determinants. Our purpose was to explore more fully some physiologic correlates and determinants of the skin cholesterol content in subjects not yet treated with lipid-lowering medications.

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Sixty patients were enrolled: 8 were healthy volunteers, 47 were patients without overt vascular disease who were referred to the Lipid Clinic for evaluation of hyperlipidemia and primary prevention treatment, and 5 were patients with known vascular disease who were referred to the Atherosclerosis Reversal Clinic and had not yet been treated with lipid-lowering agents. Subjects underwent history and physical examinations and were subjected to skin cholesterol testing on the palm of each hand.[1] Using the Cholesterol 1,2,3 system (Toronto, Ontario, Canada), a single drop of a synthetic copolymer conjugated with digitonin and horseradish peroxidase is placed on the cleaned skin surface of the hypothenar eminence and allowed to incubate for 1 minute. After blotting off unbound material, indicator solution is applied that reacts with the bound horseradish peroxidase to provide a blue hue that is read with a hand-held spectrophotometer. Digitonin is expected to complex strongly with unesterified cholesterol or phytosterols (e.g., β-sitosterol), but in the stratum corneum, cholesterol is by far the dominant lipid. Fasting total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, apolipoprotein B, lipoprotein(a) (Lp(a)), C-reactive protein, and homocysteine were measured at St. Paul's Hospital. E-selectin, vascular adhesion molecule (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) were measured at Baylor College of Medicine. von Willebrand factor was measured at Vancouver Hospital. A 10-year risk calculation for coronary heart disease was performed for each patient using the Framingham risk tables.[2] The protocol was approved by the Institutional Review Board of the University of British Columbia and all patients provided informed consent.

Variability in the quantitation of skin cholesterol was assessed by comparing determinations from the right and left hands of participants, but all subsequent statistical analyses used the mean skin cholesterol value obtained from both hands. All parameters were analyzed on a univariate and multivariate basis. Dichotomous variables were initially analyzed using *t* tests. Categorical variables with >2 levels were analyzed using analysis of variance. Forward step-wise

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TABLE 1 Clinical Characteristics of Study Population (n = 60)

Variable	Number (%)
Men	29 (48%)
Age (yrs ± SD)	50 ± 12
Body mass index (kg/m^2 ± SD)	27 ± 5
Systolic blood pressure (mm/Hg ± SD)	120 ± 15
Diastolic blood pressure (mm/Hg ± SD)	75 ± 12
Systemic hypertension (history)	10 (17%)
Myocardial infarction (history)	3 (5%)
Cigarette smoker	2 (3%)
Family history of CHD	4 (7%)
Aspirin use	9 (15%)
β-blocker use	4 (7%)
ACE inhibitor or ARB use	2 (3%)
Diuretic use	4 (7%)
10-yr Framingham risk (mean % ± SD)	8 ± 6%

ACE = angiotensin-converting enzyme; ARB = angiotensin II type 1 receptor blocker.

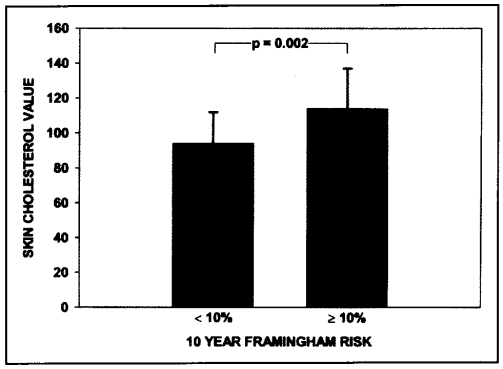

FIGURE 1. Mean value of skin cholesterol test result of patients with a <10%/10-year Framingham risk and with a ≥10%/10-year risk.

TABLE 2 Physiologic Correlates of Skin Cholesterol Measurement as Assessed by Univariate Analysis

	Correlation Coefficient	p Value
Framingham risk calculation (%)	0.38	0.003
Systolic blood pressure	0.30	0.018
Total cholesterol	0.31	0.019
ICAM-1	0.33	0.020
LDL	0.29	0.026

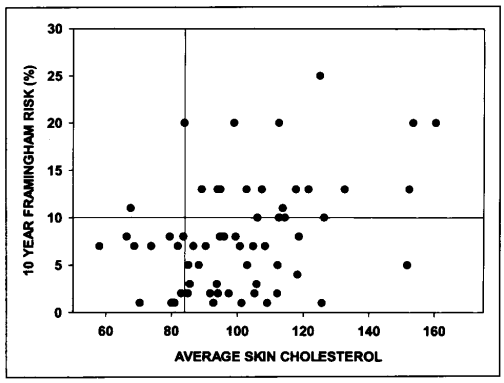

FIGURE 2. Relation between individual values of the skin cholesterol test *(x axis)* and 10-year Framingham risk *(y axis).*

regressions were done. Analyses were undertaken with and without imputation of missing data resulting from difficulties in retrieving stored blood samples for the analysis of ICAM-1, VCAM-1, E-selectin (9 patients), and von Willebrand factor (1 patient).[3] In addition, data inspection revealed 3 patients who had outlier values. One patient with probable homozygous familial hypercholesterolemia had extremely high levels of total cholesterol, LDL, and apolipoprotein B. A second patient had an extremely high value of C-reactive protein and VCAM-1. A third patient had an extremely high level of ICAM-1. They were excluded from the reported analyses, except where indicated.[4] Statistical tests were considered significant at the $p < 0.05$ level. Values are reported as mean ± 1 SD.

The mean skin cholesterol values ranged from 58 to 160 skin cholesterol units (U). The mean difference of measurements between the right and left hand was 5 U. The mean absolute difference was 21 U. The precision (SD of mean difference) was 28 U. Values between hands correlated with an $r = 0.42$ ($p = 0.001$, SEE 23 U). Data on file from the company (IMI International Medical Innovations, Toronto, Ontario, Canada) indicates a 3% to 11% within-day, day-to-day, and reagent batch-to-reagent batch variability in subjects with normal and significantly elevated levels of cholesterol.

The characteristics of the population are listed in Table 1. Significant and positive correlations (i.e., skin cholesterol measurement increases as value of physiologic parameter increases) were noted with respect to Framingham risk calculation, systolic blood pressure, total cholesterol, LDL cholesterol, and ICAM-1 (Table 2). Other parameters that were close to being significantly correlated included: gender (men had a tendency toward a lower value of skin cholesterol, $p = 0.079$), age ($p = 0.076$), triglycerides ($p = 0.062$), total cholesterol/HDL ($p = 0.053$), apolipoprotein B ($p = 0.064$), C-reactive protein ($p = 0.069$), and VCAM-1 ($p = 0.094$). Stepwise multivariate analyses were undertaken with and without imputation of missing data as previously indicated. Using either approach, a relation was seen with the Framingham risk calculation ($p \leq 0.0014$). As noted in the univariate analyses, gender also appeared to be a partial determinant of the skin cholesterol measurement ($p = 0.0077$) but only when missing data were not imputed. Interestingly, 2 inflammatory markers (E-selectin and ICAM-1) were also correlated with the skin cholesterol measurement ($p = 0.0231$ and $p = 0.0002$, respectively), but only when missing data were imputed.

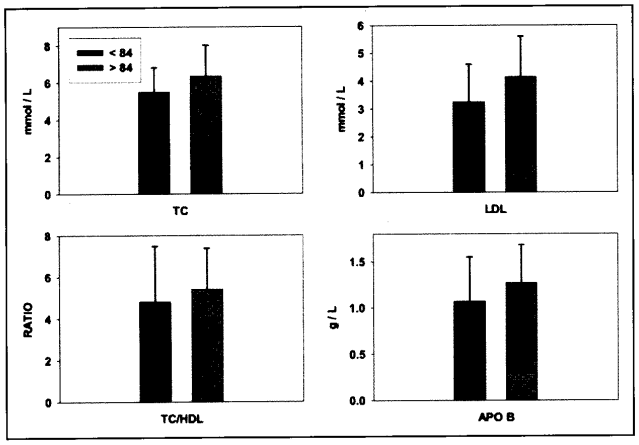

FIGURE 3. Mean values for total cholesterol (TC), LDL, apolipoprotein B (apo B, and the TC/HDL ratio based on skin cholesterol <84 or ≥84.

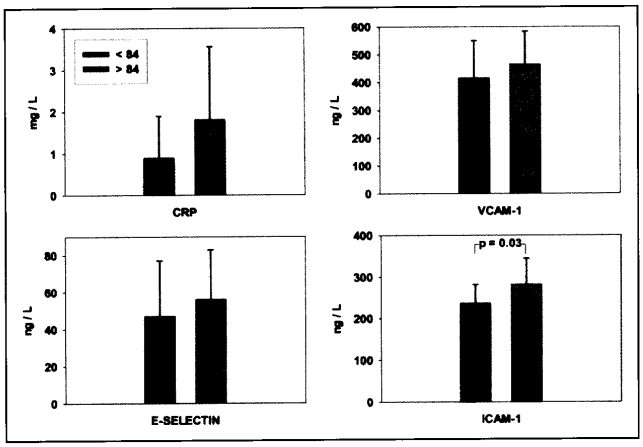

FIGURE 4. Mean values for inflammatory markers (VCAM-1, endothelial cell selectin [E-selectin], ICAM-1, and C-reactive protein [CRP]) based on skin cholesterol <84 or ≥84.

Figure 1 shows the mean skin cholesterol value between those with a 10-year Framingham risk <10% (94 ± 18) compared with those with a higher risk (114 ± 23, p = 0.002).

Figure 2 shows the relation between the skin cholesterol score and the 10-year Framingham risk calculation for individuals. Almost all patients with a risk >10% had a skin cholesterol value above a threshold of 84. Only 1 patient with a risk of 12% had a skin cholesterol value below this threshold. Based on the threshold value of 84, we were unable to detect a significant preponderance of men or women in either the high or low categories of skin cholesterol. In addition, the mean values were not different between men and women (106 ± 24 vs 96 ± 19, men vs women, respectively; p = 0.08).

Figure 3 shows that patients with a skin cholesterol value of ≥84 had a higher mean values of total cholesterol, LDL, total cholesterol/HDL, and apolipoprotein B. Similarly, Figure 4 shows that patients with skin cholesterol values of ≥84 had mean increases in all inflammatory markers, but only ICAM-1 was significantly elevated. Figure 5 shows the same result even when only patients with a Framingham risk of <10%/10 years were analyzed. All of these relations were the same irrespective of whether outlier data were included or not. Imputed data were not used for any of these subset analyses.

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This pilot study was undertaken to determine physiologic correlates of this novel test in a population that was not treated with lipid-lowering agents. Our extensive analyses, using multiple statistical approaches, show that the skin cholesterol measurement is predominantly correlated with the integrated risk of future events, as calculated using the Framingham approach. The Framingham approach is based on age- and gender-specific weighting of the traditional risk factors of blood pressure, cholesterol, HDL, presence or absence of diabetes, and whether patients smoke or not.[2] Some of these constituent determinants of the Framingham risk calculation were also identified as univariate determinants of the skin cholesterol measurement (Table 2). The importance of the correlation with systolic blood pressure is unclear because most pressures were within the normal range and multivariate analysis failed to identify this as an independent correlate of the skin cholesterol value. Although gender was identified as a correlate, we were unable to detect significant differences in the mean values of skin cholesterol or the frequency of abnormal skin cholesterol values between men and women. More important, however, was the demonstration that the skin cholesterol test correlated with inflammatory markers of vascular disease, a dimension of risk assessment that is not a component of the Framingham risk stratification process. The relation with inflammatory markers was statistically strongest with respect to ICAM-1. However, the mean values of all the inflammatory markers measured in this study were generally higher in pa-

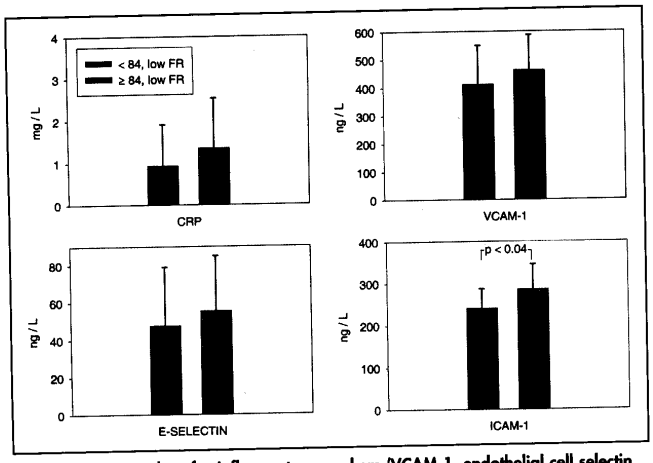

FIGURE 5. Mean values for inflammatory markers (VCAM-1, endothelial cell selectin [E-selectin], ICAM-1, and C-reactive protein [CRP]) based on skin cholesterol <84 or ≥84 in patients with a calculated Framingham risk of <10%/10 years (low FR).

tients with skin cholesterol values ≥84 (Figure 4). This relation remained even in those patients with a Framingham risk of <10% (Figure 5). These observations are important because in recent years a major interest in markers of inflammation has emerged; such markers appear to predict cardiovascular events.[5,6]

In summary, this novel skin cholesterol test reflects calculated Framingham risk but is also determined to some extent by circulating markers of inflammation, particularly ICAM-1. Thus, the test appears to reflect 2 important and independent determinants of cardiovascular risk.

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