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Skin tissue cholesterol is not related to vascular occlusive disease

Markus Reiter^a, Susan Wirth^a, Ali Pourazim^a, Stefan Puchner^b, Mehrdad Baghestanian^a, Erich Minar^a and Robert A Bucek^{a,b}

Abstract: The objective of the present study was to evaluate the role of skin tissue cholesterol (SkinTc) in predicting the presence of atherosclerosis. SkinTc concentrations were determined in 318 consecutive patients by using the non-invasive PREVU POC Skin Sterol Test. Additionally, a complete lipid status and cardiovascular risk profile according to the PROCAM and Framingham scores as well as an evaluation by carotid duplex sonography and ankle-brachial blood pressure index testing was obtained from all patients. SkinTc concentrations did not differ significantly among patients suffering from cerebrovascular disease (CVD) and peripheral arterial disease (PAD) compared to the corresponding control groups and among patients with a calculated cardiovascular risk > 10% in 10 years compared to patients with a risk < 10% (all $p > 0.05$). Additionally, SkinTc concentrations were not significantly higher in the 245 patients with at least one documented atherosclerotic disease compared with the remaining 73 patients without evidence of atherosclerosis. In conclusion, SkinTc concentrations determined by the PREVU POC Skin Sterol Test are not related to the presence of CVD and PAD or to an elevated cardiovascular risk, indicating that this parameter cannot be used as a reliable indicator of atherosclerosis.

Key words: atherosclerosis; cholesterol; Framingham; PREVU POC Skin Sterol Test; PROCAM; skin tissue cholesterol

Introduction

The Framingham Heart Study defined the traditional cardiovascular risk factors such as heredity, sex, age, smoking, arterial hypertension, glucose intolerance, obesity, physical inactivity and high levels of serum lipids.¹ The National Cholesterol Education Program has made extensive use of Framingham data for preventing coronary heart disease (CHD), especially by controlling high serum cholesterol levels, which has been demonstrated to profoundly reduce cardiovascular events and mortality.^{2–5}

However, of the 1.5 million heart attacks and 600 000 strokes that occur in the USA each year, almost half will affect apparently healthy men and women with normal or even low serum cholesterol levels.⁶ In an effort to better predict cardiovascular events, the

determination of skin tissue cholesterol (SkinTc) as a marker for deposited extracellular cholesterol has been suggested to be a further independent cardiovascular risk factor.^{7–15} Previous data indicated a relation between higher concentrations of SkinTc and the presence of CHD, but the reported SkinTc concentrations differed significantly between the studies and suffered from high standard deviations and high interquartile ranges.^{10–15} Additionally, a further study reported a missing correlation between SkinTc and high-sensitive CRP (hs-CRP) levels and the history of CHD, cerebrovascular disease (CVD) and peripheral arterial disease (PAD).¹⁶ It also has to be pointed out that the differences of SkinTc concentrations between patients with CHD and the corresponding controls were in the range of the reported with-in day and day-to-day precision and the reported natural fluctuation.^{10–15,17} Consequently, the value of SkinTc as a cardiovascular risk marker may be overrated and created by the natural variation of this parameter. A further limitation of the previous trials is that the authors provided no information about the general vascular status of the patients, which may have a major impact on SkinTc concentrations.^{10–15}

Therefore, we performed a prospective trial to clarify the role of SkinTc in cardiovascular risk assessment and evaluated the correlation of SkinTc with

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documented CVD and PAD as well as with the calculated cardiovascular risk based on the Framingham and PROCAM risk scores.^{18–20}

Methods

Consecutive outpatients, referred to the department of angiology of a university clinic for evaluation of CVD or PAD were eligible for the present prospective trial. Patients were admitted for the assessment of their general vascular status (i.e. owing to recently diagnosed arterial hypertension or diabetes mellitus, carotid bruit or carotid pulse asymmetry or weakness, leg pain to exclude or confirm intermittent claudication, skin ulceration or peripheral pulse deficit). Exclusion criteria were (1) current lipid-lowering therapy or lipid-lowering therapy within the last year; (2) age < 18 years; (3) pregnancy; (4) psoriasis or eczema on either hand; and (5) recent use (within 24 hours prior to testing) of a topical medication or cream or lotion on either hand. The study protocol was approved by the local ethics committee and written informed consent was obtained from all patients.

At admission, a standardized questionnaire was used to record the patient's medical history with a special focus on symptomatic cardiovascular disease, drug intake, arterial blood pressure (Riva-Rocci) and body mass index (BMI).

Blood samples

Antecubital venous blood samples were obtained for the determination of total plasma cholesterol (TC), serum low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), glucose and glycosylated hemoglobin A1c (HbA1c) levels. An Olympus AU5400 analyzer with standardized Olympus System Reagents (Olympus Diagnostica GmbH, Hamburg, Germany) was used for the determination of lipid levels, a Menarini-Arkray HA 8140 hemoglobin (Hb) A1c analyzer (Menarini Diagnostics, Florence, Italy) was used for the measurement of HbA1c levels.

PREVU POC Skin Sterol Test

The PREVU POC Skin Sterol Test (PreMD Inc., Toronto, Canada) was performed on the palmar surface of each hand as described recently.^{10–17} The subsequent statistical analysis used the mean value obtained from both hands. The test required no prior fasting and after vigorously cleaning the palm of the hand with an alcohol swab, the photometric reaction of a detector (Digitonin horseradish peroxidase)/indicator (tetramethylbenzidine hydrogen peroxide solution) system was analyzed by a MD22 Spectrophotometer (X-Rite, Inc., Michigan, USA) interfaced with a computer (Sony Computer, Tokyo, Japan) generating a numeric

absorber units value for SkinTc. Negative and positive controls applied to the palm in parallel with the detector solution served to monitor test performance. In the pre-study phase, we observed a within-day imprecision of 3.8% and a between-day imprecision of 8.6% for the right hand and 4.3% for the left hand.^{16,17}

Cardiovascular screening tests and definition of vascular disease groups

Carotid duplex ultrasound was performed by experienced vascular technicians on an Acuson XP 10 scanner (Acuson, Mountain View, CA, USA) with a 5-MHz linear probe according to the principles of the Society of Radiologists in Ultrasound concerning the quantification of stenosis of the extracranial carotid artery and the proposals of Nicolaides et al.²¹ According to previously published data, our duplex laboratory achieves an excellent agreement of 96% ($\kappa = 0.85$) with angiography and was therefore used to reliably classify the macrovascular CVD in three groups: group 1 (no plaques visible); group 2 (< 50% stenosis, hemodynamically not significant); and group 3 (> 50% stenosis, hemodynamically significant).²² Patients with a prior history of carotid surgery or carotid artery stenting were classified in group 3 irrespective of the results of duplex ultrasound.

Complete peripheral pulse status and determination of the ankle-brachial index (ABI) (calculated as ratio of the systolic arterial ankle pressure to brachial artery pressure) were obtained in all patients. PAD was classified in three groups: group 1 (ABI index > 0.9 at rest, regular pulse status); group 2 (ABI index < 0.9 at rest; asymptomatic patients); and group 3 (ABI index < 0.9 at rest; patients suffer from intermittent claudication or ischemic rest pain).²³ Patients with a history of peripheral vascular surgery or percutaneous transluminal angioplasty and/or stenting were classified in group 3. Diabetic patients with incompressible arteries due to mediasclerosis, which precludes accurate pressure measurements, were classified in group 2 if asymptomatic, symptomatic diabetic patients were classified in group 3.

The total percent risk of heart disease in 10 years for the Framingham risk score and the total PROCAM points were calculated as described previously.^{19,20} All patients were classified in three groups separated for each score: group 1 (low, < 10% estimated risk); group 2 (average, 10–20% estimated risk); and group 3 (high, > 20% estimated risk).^{19,20}

Definition of cardiovascular risk factors

Individuals who smoked regularly during the previous 12 months were classified as current smokers. Arterial hypertension was classified in patients receiving anti-hypertensive medication or in patients with systolic blood pressure values ≥ 140 mmHg or diastolic values ≥ 90 mmHg according to the guidelines of the

Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.²⁴ Diabetes mellitus was classified in patients with a HbA1c level > 6% accompanied by a fasting blood glucose level greater than 110 mg/dl or in patients with a pathologic oral glucose tolerance test and was assumed to be present in patients with a history of treatment with insulin or oral hypoglycemic agents.^{25,26} Hyperlipidemia was classified in patients with a fasting total serum cholesterol > 200 mg/dl, a serum LDL-C value greater than 130 mg/dl or a serum TG value of greater than 172 mg/dl according to the local standard values.

Statistical analysis

A sample size calculation (based on an estimated power of 0.8) assumed a standard deviation (SD) of 30U concerning SkinTc and revealed a minimal sample size of 289 patients; we assumed a safety margin of an additional 10%. Statistical analysis was performed by using SPSS 13.0 (SPSS Inc, Chicago, IL, USA). Numeric values were tested for normal distribution and are stated as mean \pm standard deviation. Student's *t*-test was used to evaluate differences between groups and correlation analysis used Pearson's correlation coefficient (*r*). Univariate ANOVA analysis was used to assess differences of SkinTc concentrations between the three stages of CVD, PAD and the Framingham and PROCAM calculated cardiovascular risk. Regression analyses were used to assess influence factors on the presence of atherosclerosis and to evaluate the influence of the presence of atherosclerotic disease on SkinTc. A general linear regression analysis included traditional cardiovascular risk fac-

tors (age, sex, diabetes mellitus, arterial hypertension, smoking, TC, LDL-C) and the presence of PAD and CVD with SkinTc as the dependent variable. A logistic regression analysis was adapted in a forward manner, including traditional cardiovascular risk factors with the presence of atherosclerosis as the dependent variable. A *p*-value < 0.05 was considered significant.

Results

In total, 318 consecutive patients were included in the present trial. Their baseline demographic data and laboratory results distributed to the three stages of CVD are shown in Table 1. The mean SkinTc concentration of the total study population was 107.7 ± 23.8 U. Univariate analyses revealed no significant correlation of SkinTc with traditional cardiovascular risk factors such as age, BMI, TC, HDL-C, LDL-C and TG and no significant differences for sex, diabetes mellitus, arterial hypertension, hyperlipidemia and smoking concerning SkinTc concentrations (all *p* > 0.05).

The *t*-test analyses demonstrated no significant differences in SkinTc concentrations between the patients with (groups 2 and 3) and without (group 1) CVD or PAD (*p* > 0.05). In addition to that, we failed to detect significant higher SkinTc concentrations in patients with a cardiovascular risk > 10% in 10 years compared with patients with a risk < 10% in 10 years according to the Framingham and PROCAM scoring systems (all *p* > 0.05); detailed results of the ANOVA analyses are stated in Table 2.

A comparison was made of the 245 patients with at least one documentation of atherosclerotic disease

Table 1 Baseline demographic data and laboratory results of the study distributed according to the three-group system for CVD.

| | Group 1 <i>n</i> = 105 | Group 2 <i>n</i> = 143 | Group 3 <i>n</i> = 70 |
|----------------------------------|---------------------------|---------------------------|--------------------------|
| Sex | | | |
| male | 48 (45.7%) | 87 (60.8%) | 44 (62.9%) |
| female | 57 (54.3%) | 56 (39.2%) | 26 (37.1%) |
| Mean age (years) | 48.6 \pm 12.8 | 66.2 \pm 10.5 | 73.9 \pm 9.9 |
| Mean BMI (kg/m ²) | 26.1 \pm 4.7 | 26.8 \pm 3.9 | 26.4 \pm 4.3 |
| Diabetes mellitus | 11 (10.5%) | 56 (39.2%) | 33 (47.1%) |
| Arterial hypertension | 35 (33.3%) | 93 (65%) | 63 (90%) |
| Hyperlipidemia | 75 (71.4%) | 114 (79.7%) | 51 (72.9%) |
| Current cigarette smoking | 31 (29.5%) | 54 (37.8%) | 20 (28.6%) |
| History of myocardial infarction | 3 (2.9%) | 10 (7%) | 10 (14.3%) |
| History of cerebral infarction | 0 | 12 (8.4%) | 14 (20.0%) |
| TC (mg/dl) | 221.7 \pm 37.2 | 245.0 \pm 100.7 | 220.5 \pm 48.3 |
| LDL-C (mg/dl) | 132.4 \pm 33.5 | 141.5 \pm 44.0 | 134.0 \pm 38.6 |
| HDL-C (mg/dl) | 61.6 \pm 15.9 | 58.8 \pm 16.0 | 53.5 \pm 18.5 |
| TG (mg/dl) | 154.9 \pm 144.5 | 215.9 \pm 348.2 | 164.7 \pm 97.3 |
| SkinTc (U) | 106.9 \pm 21.5 | 107.4 \pm 25.3 | 109.8 \pm 24.1 |

BMI, body mass index; TC, total cholesterol; LDL-C, serum low-density lipoprotein cholesterol; HDL-C, serum high-density lipoprotein cholesterol; TG, serum triglycerides; SkinTc, skin tissue cholesterol; U, units.

Table 2 Distribution of CVD, PAD and the PROCAM and Framingham scores stated with the corresponding SkinTc concentrations.

| | <i>n</i> | SkinTc concentration (U) ± SD |
|----------------------------------|-------------|-------------------------------|
| CVD (all <i>p</i> > 0.05) | | |
| Group 1 | 105 (33.0%) | 106.9 ± 21.5 |
| Group 2 | 143 (45.0%) | 107.4 ± 25.3 |
| Group 3 | 70 (22.0%) | 109.8 ± 24.1 |
| PAD (all <i>p</i> > 0.05) | | |
| Group 1 | 173 (54.4%) | 107.1 ± 22.3 |
| Group 2 | 44 (13.8%) | 105.2 ± 26.0 |
| Group 3 | 101 (31.8%) | 110.7 ± 25.5 |
| PROCAM (all <i>p</i> > 0.05) | | |
| < 10% in 10 years risk | 166 (52.2%) | 108.1 ± 21.8 |
| > 10% to < 20% in 10 years risk | 90 (28.3%) | 108.7 ± 26.7 |
| > 20% in 10 years risk | 62 (19.5%) | 105.5 ± 24.6 |
| FRAMINGHAM (all <i>p</i> > 0.05) | | |
| < 10% in 10 years risk | 118 (37.1%) | 107.1 ± 21.5 |
| > 10% to < 20% in 10 years risk | 110 (34.6%) | 107.7 ± 23.8 |
| > 20% in 10 years risk | 90 (28.3%) | 108.8 ± 26.7 |

SkinTc, skin tissue cholesterol; U, units; CVD, cerebrovascular disease; PAD, peripheral arterial disease.

(PAD or CVD 2 and 3) and/or a cardiovascular risk > 10% in 10 years and the remaining 73 patients, who were classified in group 1 for PAD and CVD and had a cardiovascular risk < 10% in 10 years and therefore exhibited no evidence or increased risk of atherosclerotic disease and served as the control group. SkinTc was not significantly higher in patients with atherosclerotic disease (108.0 ± 24.7 U vs 106.9 ± 20.5 U; *p* > 0.05). Our results were confirmed by a general linear regression analysis, which detected no significant influence factor on SkinTc concentrations, based on the inclusion of the following parameters: sex, age, BMI, TC, LDL-C, HDL-C, TG, arterial hypertension, diabetes mellitus, smoking, the presence of PAD and the presence of CVD (all *p* > 0.05). In addition to that, our analyses showed that the 23 patients with a history of myocardial infarction exhibited no significantly higher SkinTc concentrations compared with the remaining individuals (116.5 ± 28.7 U vs 107.1 ± 23.3 U; *p* > 0.05).

In order to rule out a systematic failure of our study cohort we performed additional analyses, which demonstrated that our data are in accordance with the Framingham risk profile because the patients in the atherosclerosis group were significantly older and had significantly higher levels of TC, TG and LDL-C (all *p* < 0.005) and a higher incidence of arterial hypertension (176 vs 15 patients), diabetes mellitus (97 vs three patients) and smoking history (91 vs 14). A logistic regression analysis, which included sex, age, diabetes mellitus, arterial hypertension, smoking, TC and LDL-C confirmed this finding and demonstrated a significant influence of all included parameters (with the exception of LDL-C) on the presence of atherosclerosis (all *p* < 0.05).

Discussion

The major risk factors included in the Framingham risk score account for > 80% of the excess risk for premature CHD.²⁷ An abnormal ABI and the visualization of carotid artery plaques by ultrasound add to the information provided by traditional risk assessment.^{18,28} The determination of SkinTc has been suggested as an adjunct to the traditional cardiovascular risk factor testing.^{1,10–15,19} Previous trials suggested a correlation between SkinTc and the presence of CHD, but these trials provided no further information about the general vascular status of their patients.^{10–15} Atherosclerosis is of course not limited to the coronary arteries and a cardiovascular risk predictor additionally includes the atherosclerotic status of the remaining arterial system.

The present trial is the first providing a complete objectively determined vascular status of the study cohort by evaluating the patients by carotid duplex sonography, the determination of the ABI and standardized cardiovascular risk scores. We demonstrated that SkinTc concentrations are not higher in patients with documented vascular disease and/or an increased cardiovascular risk compared to patients without any evidence of atherosclerotic disease. Additionally, separated ANOVA analyses of each vascular disease group and both cardiovascular risk groups revealed no significant difference between patients without (group 1), with limited (group 2) and with advanced (group 3) disease. Even patients with passed myocardial infarction did not exhibit elevated SkinTc concentrations.

Our data is in accordance with a previously published trial¹⁶ reporting a missing correlation between

SkinTc concentrations and a history of cardiovascular events, but which challenges the remaining data and therefore has to be analyzed more precisely.^{10–15} A systematic technical failure in the execution of the skin test does not seem a probable explanation because the SkinTc concentrations and standard deviations of our study are comparable with previously published data.^{10–17} A misinterpretation based on our study population is also not likely because traditional cardiovascular risks were significantly associated with the presence of atherosclerosis in our patients, indicating that a representative study cohort was investigated. Additionally, the number of patients included in our study was based on a detailed sample size calculation in order to provide analysis with high statistical power. Therefore, we reviewed the previous trials in detail.^{10–15}

It has to be pointed out that the SkinTc concentrations of patients and controls varied considerably among the studies.^{10–15} While Vaidya et al reported on a median SkinTc concentration of 91 U (IQR 24) for patients exhibiting coronary artery calcium (CAC) versus 83 U (IQR 20) for controls, Sprecher et al showed mean SkinTc levels of 124 ± 30 U for patients with coronary artery stenosis versus 118 ± 30 U for patients without stenoses and mean SkinTc levels of 127 ± 29 for patients with a history of myocardial infarction versus 120 ± 20 with those without.^{12–14} A further study suggested a threshold value of 84 U for an increased 10-year Framingham risk.¹¹ A potential explanation for the variation of SkinTc concentrations might be that most of these trials investigated a relatively small number of patients. Vaidya and co-workers demonstrated the correlation between SkinTc and CAC only in a small subgroup analysis of 99 white patients and failed to detect a significant correlation in the remaining 123 patients – all African-Americans – of their study cohort.¹³ In addition to that, the significant differences reported in their study diminished after hypertension was adjusted for using logistic regression.¹³ Tzou et al included 81 patients and demonstrated an increased IMT not until the highest quartile of SkinTc, but the low statistical power of these results has to be considered in the interpretation of this study reporting data from small groups.¹⁵ The findings of Mancini et al was based on the evaluation of an inhomogeneous group of 60 individuals: eight healthy volunteers, five patients with known vascular disease and 47 patients with unknown vascular status.¹¹ Zawydiwski et al investigated 111 individuals without known CHD, but only 15 patients had a positive stress test and the reported SkinTc concentrations exhibited a wide range for patients (median 0.059 U, range 0.031–0.166 U) and controls (median 0.040 U, range 0.003–0.118 U).¹⁰

Our literature research detected only two statistically powerful publications, both by Sprecher et al.^{12,14} However, only one of these trials reports original

data about a correlation between SkinTc and the presence of coronary artery stenosis.¹² The second article, describing a correlation between SkinTc and a history of myocardial infarction, reports findings corresponding with the first study; obviously for the same patient cohort, so no additional information has been provided.^{12,14} A further major limitation of these two studies and the majority of the previous studies is the use of the SkinTc concentrations of one hand instead of the mean value of both hands for their analyses.^{10,12–15} This fact is of special interest because only a moderate correlation between the right and the left hand could be observed in our study ($r = 0.55$) and in two further studies ($r = 0.42$ and $r = 0.57$).^{11,16}

Additionally, as mentioned above, most studies including the data of Sprecher et al suffered from a limited description of the study population.^{10–15} None of the trials reported on concomitant vascular occlusive disease. Only Vaidya et al and Tzou et al evaluated the patients by carotid ultrasound, but only focused on the determination of the IMT and provided no information about potential concomitant carotid stenoses even though the investigated population had a mean age of 66 years and 56 years and the presence of advanced CVD was therefore likely.^{13,15} Data about the potential presence of PAD are missing in both publications. Thus, the incomplete evaluation of the study populations may be a potential explanation for the inconsistent findings of the previous trials because patients without evidence of CHD potentially suffered from concomitant CVD or PAD and therefore may have been wrongly classified as ‘healthy controls’.^{10–15} The only study providing information about concomitant vascular diseases failed to detect a correlation between SkinTc and a history of cardiovascular events.¹⁶

Finally, a significant natural fluctuation of SkinTc concentrations – which has been observed during a period of 6 months and constitutes about 12% – and a within-day and day-to-day precision of the skin test of 4–11% and 7–9% have to be considered in the interpretation of the previous trials and may have influenced or created their results.^{10–17}

A limitation of the present trial was that a direct visualization of the coronary arteries by angiography is missing. We used the 10-year risk according to the PROCAM and Framingham scoring systems instead of an invasive procedure in order to reflect cardiovascular disease by a risk calculation because these scores accurately predict coronary events with an area under the receiver-operating characteristics curve of about 80%.¹⁹

In conclusion, SkinTc concentrations determined by the PREVU POC Skin Sterol Test are neither related to the presence of CVD or PAD nor to an elevated cardiovascular risk, indicating that this parameter cannot be used as a reliable indicator of atherosclerosis.

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Conflicts of interest

There are no conflicts of interest.

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