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Skin tissue cholesterol assay correlates with presence of coronary calcium

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Abstract

Background: Keratinocytes maintain cholesterol homoeostasis using LDL and HDL-scavenger receptor pathways implicated in atherosclerosis. We tested whether skin tissue cholesterol (SkTC) is associated with subclinical atherosclerosis, assessed using carotid intima-medial thickness (IMT) and coronary artery calcification (CAC).

Methods: Two hundred and twenty two subjects were recruited from the Baltimore site of the Multi-Ethnic Study of Atherosclerosis (MESA). Baseline MESA examination included fasting blood collection, IMT measurement by B-mode ultrasound and CAC determination by helical CT. SkTC was measured using the Cholesterol 1, 2, 3 kit (IMI Inc., Canada).

Results: SkTC was significantly higher if CAC was present in Caucasians ($p = 0.04$) but not African–Americans. SkTC was not significantly correlated with IMT in either group. Using multiple logistic regression adjusting for age, sex and serum lipids, a 1-standard deviation higher SkTC was associated with 187% higher odds of the presence of CAC in Caucasians ($p < 0.01$) but no significant difference in odds for African–Americans. In subjects in whom CAC was present, the extent of CAC was not related to the SkTC in multiple linear regressions.

Conclusions: SkTC was associated with the presence of CAC independently of serum lipids/lipoproteins in Caucasians but not African–Americans. SkTC may provide a useful indicator of subclinical atherosclerosis in Caucasians.

Keywords: Subclinical atherosclerosis; Skin tissue cholesterol; Risk factors; Coronary calcium

1. Introduction

Coronary artery disease (CAD) is the leading cause of death in Western countries. Identifying individuals with early subclinical atherosclerosis may better target individuals for effective risk reduction. Serum cholesterol, which is widely used to identify high-risk individuals, does not directly assess the extent of cholesterol deposition in tissues. The measurement of skin tissue cholesterol (SkTC) may provide this additional information. The Institute of Physicochemical Medicine in the former Soviet Union originally developed a non-invasive assay of SkTC to screen populations for atherosclerotic risk [1]. The methodology has now been standardized by International Medical Innovations (IMI) Corporation and evaluated in studies involving relatively small and well-characterized groups of subjects [2]. This colorimetric technique is reproducible, painless and quick to perform. Studies have shown a correlation between SkTC and Framingham risk prediction score [3] and angiographically defined CAD [4]. The aim of this study is to evaluate whether this technique can discriminate individuals with

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subclinical atherosclerosis, as assessed by coronary artery calcium (CAC) and carotid intima-medial thickness (IMT) within subjects recruited from the Multi-Ethnic Study of Atherosclerosis (MESA) [5].

2. Methods

MESA is a federally funded multi-center, longitudinal cohort study of the factors that influence the progression of mild subclinical cardiovascular disease (CVD) to severe subclinical and clinical CVD in a multi-ethnic group of subjects [5]. Briefly, men and women aged 45–85 years, without a history of clinical CVD completed the baseline examination between July 2000 and August 2002. Of these, 222 consecutive Caucasian and African–American subjects were recruited at the Johns Hopkins University site for this ancillary study. All subjects approached agreed to participate and gave informed consent as approved by the MESA Ancillary Studies Committee, Steering Committee, NHLBI Review Board and the local Institutional Review Board. The baseline MESA examination included questionnaires, verification of medication history, blood pressure determination, fasting blood collection, carotid IMT by ultrasound and CAC determination by helical CT, among other examinations. These 222 subjects also had measurements of SkTC using the Cholesterol 1, 2, 3 [6], a patented, non-invasive, point-of-care SkTC assay.

2.1. Skin tissue cholesterol assay

A digitonin-binding assay was used to determine SkTC level. Cholesterol is by far the most abundant of digitonin-binding unesterified sterols in the stratum corneum. A digitonin-horseradish peroxidase conjugate was added to a well in a pad applied to the palmar skin. After 1 min, the well was blotted dry and peroxidase indicator solution was added and incubated for 2 min. The colored hydrolysis product catalyzed by bound peroxidase was read using a hand held spectrophotometer. Adjacent wells in the pad served as reaction controls. The depth of binding of the conjugate to epidermis has been shown to be less than 0.3 mm and restricted to the epidermis [7]. The detector had a linear range of 0.02–2.0 mg/ml cholesterol in vitro. A laptop computer program provided step-by-step instructions and ensured accurate timing of each step of the reaction. The SkTC colorimetric assay reported the hue angle in degrees on the standard HSV axes, with 65 units representing the average palmar skin hue (which does not vary by skin pigmentation) [2]. The nominal range on the colorimetric substrate was 50–200 units [2], however, only values between 60 and 150 units were used based on kit manufacturer recommendations.

2.2. Coronary calcium score

Subjects were scanned at Johns Hopkins Hospital on a Siemens S4+ Volume Zoom Helical CT scanner during a single breath hold with ECG gating according to the MESA protocol. Each subject was scanned twice on the same day. All studies were analyzed at the MESA CT reading center at Harbor UCLA. A mean Agatston CAC score was obtained from two scans. In two subjects, only one scanned score was available for analysis. The threshold for calcium detection was a minimum of four adjacent voxels (2.8 mm^2) each with brightness greater than 130 Hounsfield units.

2.3. Carotid ultrasound

Ultrasound images of the extracranial portion of the internal carotid (IC) and the distal common carotid (CC) arteries were recorded on Super VHS tape. The scans are digitized and read at the MESA Ultrasound Reading Center at New England Medical Center. The maximum IC intima-medial thickness (IMT) of the near and far walls was measured using three scan views (anterior oblique, lateral oblique and posterior oblique) of both the left and right arteries. A mean of these 12 measurements was used in the analysis. The maximum CC IMT of the near and far walls of both arteries was measured in one scan view and a mean of these four measurements was used.

2.4. Serum lipids and lipoproteins

Fasting blood samples were analyzed at the MESA core laboratory at University of Minnesota for total cholesterol (TC), HDL-cholesterol (HDL), and triglyceride (TG) levels. Levels of LDL-cholesterol (LDL) were calculated using the Friedewald formula, except for three individuals with TG levels > 400 mg/dL.

2.5. Risk factors

Subjects were considered hypertensive if their seated blood pressure was greater than 139 mmHg systolic, 89 mmHg diastolic, or if they had prescription verified history of antihypertensive medication use. Subjects were considered diabetic if their fasting blood glucose level was greater than 125 mg/dL or if they had prescription verified history of antidiabetic medication use.

2.6. Statistical analyses

The distribution of all variables was examined. IMT was log-transformed hence differences in IMT are presented as fold change. Bootstrapped analyses were used when SkTC was the dependent variable, because the right skewed SkTC distribution is not normal or lognormal. CAC was highly right skewed and included a large number of zero values. This variable was analyzed in two stages:

(1) The score was coded as a binary variable, i.e., CAC present/absent. In three subjects, only one of the two scans showed CAC to be present. These were classified

as "CAC present" in the analysis. Exclusion of these observations in supplementary analysis did not materially change either the point estimates or their statistical significance.

(2) Non-zero calcium scores were log-transformed and analyzed separately. Differences are presented as fold change.

Preliminary analyses showed that SkTC significantly interacted with ethnic background when CAC was the outcome variable. Hence, all analyses have been performed separately in Caucasians and African–Americans. The Johns Hopkins site enrolled only these racial/ethnic groups.

Differences in demographic variables between racial/ethnic groups were examined using the Student's *t*-test. Differences in SkTC distributions were tested using the Pearson's χ^2-test. Associations of SkTC with serum lipids/lipoproteins and risk factors for coronary disease were assessed using multiple linear regression using bootstrapping with 2000 samples. These analyses were adjusted for sex and age group. Serum lipids/lipoproteins and risk factors were either entered in the regression models one at a time or were all entered in a single model. Regression coefficients were considered to be significant if the bootstrapped 95% confidence intervals excluded zero (significant at the borderline if the 90% confidence interval excluded zero).

The association between IMT and SkTC was tested using Spearman correlation. The independent effects of serum lipids/lipoproteins and SkTC on IMT were determined using multiple linear regression. The pairwise associations between CAC (present/absent) and serum/skin tissue cholesterol were analyzed using the Mann–Whiney rank-sum test. CAC as a binary outcome was analyzed using multiple logistic regression. Non-zero CAC values were analyzed using multiple linear regression of log(CAC). Age group and sex were used as covariates in all regression models. LDL, being a calculated variable, was dropped when TC, HDL and TG were covariates. Since colorimetric units for SkTC and units for serum lipids are not biologically intuitive, regression coefficients are presented in units of standard deviation of the measures.

Supplementary analyses were also performed with CAC as the outcome, where the risk factors of hypertension, diabetes and smoking (ever smoker) were adjusted one at a time.

2.7. Role of funding source

The MESA Steering Committee approved the study design and collection of data, the MESA data-coordinating center maintained the master dataset, and the MESA Publications and Presentations Committee approved the decision to submit the manuscript for publication. IMI Corporation, which provided test kits, had no input in data evaluation, analysis, and preparation of this report.

3. Results

3.1. Demographics

Table 1 presents the characteristics of the subjects in the sample. African–Americans were oversampled in this study population, which reflects the predetermined ethnic distribution for the specific MESA site. African–Americans in this consecutive series of subjects were also younger than Caucasians, though the age distribution of ethnic groups is similar in the MESA cohort as a whole. The prevalence of hypertension and diabetes is as expected for this age group. African–Americans in this group have lower total serum cholesterol and SkTC levels than Caucasians. However, SkTC distributions do not differ between the two groups (Fig. 1).

Table 1
Baseline demographic characteristics of mesa skin tissue cholesterol ancillary study (2000–2002)

	Caucasian	Afr–Amer	Total	*p*-Value
N	99	123	222	–
Females	45%(45)	41%(51)	43%(96)	0.551
Age (years)	68.8 ± 8.1	63.9 ± 9.7	66.1 ± 9.4	0.0001
Hypertension	49%(49)	61%(75)	56%(124)	0.087
Diabetes	15%(15)	20% (24)[a]	18%(39)	0.366
Ever smokers	55% (54)[b]	56% (68)[c]	55%(122)	0.925
TC (mg/dL)	197.1 ± 32.4	187.1 ± 30.1	191.5 ± 31.5	0.019
LDL (mg/dL)	119.1 ± 30.4[b]	117.0 ± 27.9[a]	117.0 ± 29.0	0.605
HDL (mg/dL)	51.9 ± 14.3	48.8 ± 14.2	50.2 ± 14.3	0.104
TG (mg/dL)	130.3 ± 75.4	113.9 ± 90.4	121.2 ± 84.2	0.151
SkTC (units)	93.2 ± 18.4	87.9 ± 16.3	90.3 ± 17.4	0.024

Sex distribution, hypertension and diabetes prevalence are presented as percentage (number), all other variables are presented as mean ± standard deviation; Afr–Amer: African–American, TC: serum total cholesterol, LDL: serum low-density lipoprotein cholesterol, HDL: serum high-density lipoprotein cholesterol, TG: serum triglycerides, SkTC: skin tissue cholesterol.

[a] $n = 121$.

[b] $n = 98$.

[c] $n = 122$.

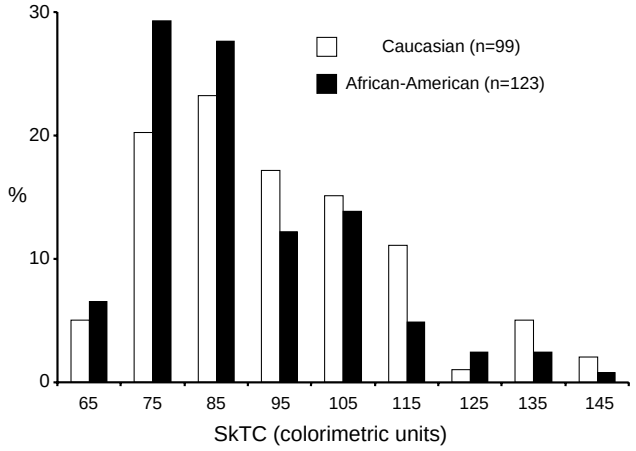

Fig. 1. Distribution of skin tissue cholesterol (SkTC) (colorimetric units), MESA Skin Tissue Cholesterol Ancillary Study (2000–2002) by racial/ethnic group. Labels on the abscissa are center values of 10-colorimetric unit bins of skin tissue cholesterol (SkTC). The difference between the two distributions is not statistically significant (Pearson's χ^2-test, $p = 0.39$).

3.2. Relationship between skin tissue cholesterol, serum lipid/lipoproteins and risk factors for coronary disease

Table 2 shows the results of bootstrapped regression analyses for associations between SkTC and serum lipids/lipoproteins as well as cardiovascular risk factors, adjusted for sex and age group. Adjusting for age and sex, SkTC is not associated with any of the serum lipids/lipoproteins or lifetime smoking status either separately or independently of the other predictors. There is an association of borderline significance of diabetes with SkTC in African–Americans ($p < 0.1$) but not Caucasians. There is statistically significant association of SkTC with hypertension in Caucasians. When all variables were added to the regression model, hypertension remained a significant predictor of higher SkTC in Caucasians. When both racial/ethnic groups were analyzed in a single regression model, hypertension continued to be a significant predictor, and there was no interaction between ethnic background and this effect.

3.3. Relationship between subclinical atherosclerosis and skin/serum lipid measures

Using Spearman rank correlation, IMT is not related to SkTC either in Caucasians (IC: $\rho = 0.15$, $p = 0.15$; CC: $\rho = 0.02$, $p = 0.81$) or African–Americans (IC: $\rho = 0.03$, $p = 0.77$; CC: $\rho = -0.05$, $p = 0.57$). The presence of any CAC was significantly associated with higher SkTC levels in Caucasians (median 91, IQR [81–105] units, versus median 83, IQR [74–94] units if CAC was absent, $p = 0.04$; IQR: interquartile range) but not in African–Americans (median 82, IQR [75–99] units, versus median 85, IQR [78–99] units if CAC was absent, $p = 0.38$).

To determine the independent association between IMT and lipid measures, two series of linear regression models were evaluated; log(IMT) measures regressed against SkTC adjusting for age and sex (Model 1), and in another model adjusting for age, sex and serum lipid/lipoprotein measures (Model 2). SkTC was not significantly associated with higher IC IMT either in Caucasians (Model 1, 3 [−2 to 9]%; Model 2, 3 [−2 to 8]% higher/10 units) or African–Americans (Model 1, 1 [−4 to 6]%; Model 2, 1 [−4 to 7]% higher/10 units). Neither was any association found between CC IMT and SkTC among Caucasians (Model 1, 0 [−2 to 2]%; Model 2, 3 [−2 to 8]% higher/10 units) or African–Americans (Model 1, 0 [−2 to 2]%; Model 2, 0 [−2 to 2]% higher/10 units).

Fig. 2 presents the results of logistic regression analysis of CAC (present/absent) in Caucasians. HDL and SkTC are significant predictors as single lipid predictors (adjusting for age and sex; open circles, dashed confidence intervals) as well as independently in multiple regression models (all lipid predictors as well as age and sex included; closed circles, solid confidence intervals). In this group 1 S.D. (14.3 mg/dL)

Table 2
Linear regression of skin tissue cholesterol (standard deviation units) on serum lipids/lipoproteins and cardiovascular risk factors (standard deviation units) stratified by race, MESA skin tissue cholesterol ancillary study (2000–2002)

Serum lipid measure	Caucasian			Afr–Amer		
	n	Single predictor β [95% CI]	Multiple predictors[a] β [95% CI]	*n*	Single predictor β [95% CI]	Multiple predictors[b] β [95% CI]
TC (/1 S.D.)	99	−0.12 [−0.36 to 0.11]	−0.12 [−0.38 to 0.13]	123	0.01 [−0.14 to 0.17]	0.04 [−0.14 to 0.23]
LDL (/1 S.D.)	98	−0.18 [−0.44 to 0.06]	–	121	0.07 [−0.11 to 0.23]	–
HDL (/1 S.D.)	99	0.14 [−0.09 to 0.40]	0.20 [−0.08 to 0.51]	123	−0.02 [−0.22 to 0.16]	−0.07 [−0.31 to 0.18]
TG (/1 S.D.)	99	−0.03 [−0.21 to 0.14]	0.05 [−0.17 to 0.29]	123	−0.06 [−0.19 to 0.21]	−0.09 [−0.28 to 0.24]
Diabetes (±)	99	−0.09 [−0.53 to 0.78]	−0.01 [−0.80 to 0.63]	121	0.41[c] [−0.07 to 0.93]	0.32 [−0.22 to 0.88]
Ever smoker (±)	98	−0.02 [−0.47 to 0.41]	−0.12 [−0.56 to 0.30]	122	−0.05 [−0.41 to 0.29]	0.02 [−0.41 to 0.43]
Hypertension[d] (±)	99	0.55[e] [0.12 to 0.97]	0.53[e] [0.15 to 0.90]	123	0.33 [−0.08 to 0.72]	0.26 [−0.18 to 0.70]

β: regression coefficients; for abbreviations and standard deviations see Table 1. All models adjusted for age and sex.

[a] $n = 98$.

[b] $n = 120$.

[c] 90% CI excludes 0.

[d] No significant interaction between ethnic group and hypertension, hypertension remains a significant predictor when the ethnic groups are analyzed in a single model.

[e] 95% CI excludes 0.

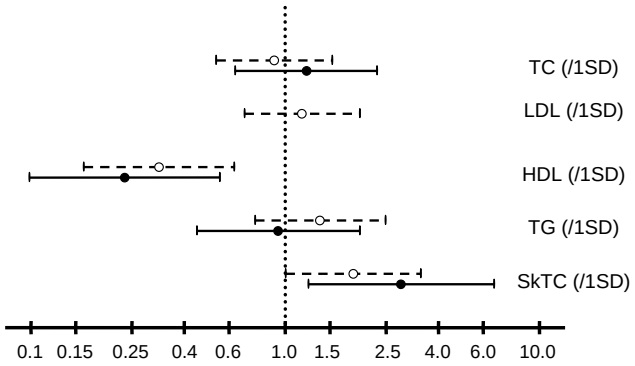

Fig. 2. Odds ratios of coronary calcium presence by skin/serum lipids/lipoproteins (standard deviation units), MESA Skin Tissue Cholesterol Ancillary Study (2000–2002) among Caucasians. $n = 99$; for abbreviations and standard deviations of variables see Table 1. Open circles, dashed 95% CI—single lipid predictor; closed circles, solid 95% CI—all lipid predictors in same model; all models adjusted for age and sex.

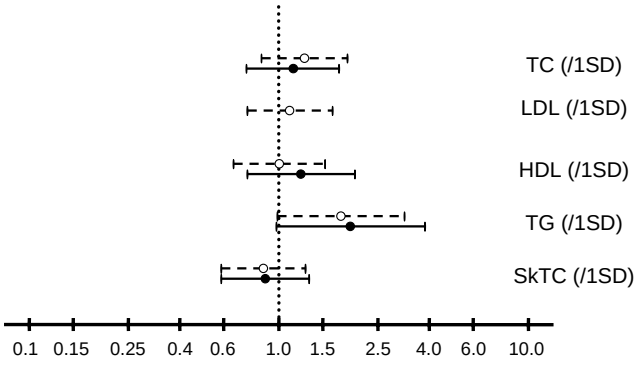

Fig. 3. Odds ratios of coronary calcium presence by skin/serum lipids/lipoproteins (standard deviation units), MESA Skin Tissue Cholesterol Ancillary Study (2000–2002) among African–Americans. $n = 123$; for abbreviations and standard deviations of variables see Table 1. Open circles, dashed 95% CI—single lipid predictor; closed circles, solid 95% CI—all lipid predictors in same model; all models adjusted for age and sex.

higher HDL was associated with 76% lower odds of CAC presence while 1 S.D. (18.4 units) higher SkTC was associated with 187% higher odds of CAC presence independent of other serum lipid/lipoprotein measures. Only serum TG was associated with borderline statistical significance (95% higher odds per 1 S.D. [84.2 mg/dL] higher TG, $p = 0.055$) with the odds of the presence of CAC in African–Americans as seen in Fig. 3. Table 3 presents the results of linear regression analysis of CAC (analyzed on a logarithmic scale) versus single and multiple lipid predictors among the 135 subjects with non-zero scores. None of the skin/serum lipid and lipoprotein measures was associated with the extent of CAC in either ethnic group.

We repeated the analyses classifying subjects as below or above the 75th percentile of CAC expected for their age and sex in the standard population as published by Hoff et al. [8]. The results of this analysis (not shown) were inferentially similar to the logistic regression from CAC present/absent presented above. In supplemental analysis, when hypertension was adjusted for using logistic regression, the association of SkTC with the presence of CAC in Caucasians was weaker and statistically significant at the borderline ($p = 0.060$).

4. Discussion

Serum cholesterol is widely used to identify individuals at high risk for atherosclerotic disease [9]. While serum cholesterol may represent the primary stimulus for atherosclerosis, a non-invasive assay of tissue deposition of cholesterol in response to this stimulus is not yet widely available. In European studies, the cholesterol content in skin biopsy specimens has been shown to correlate with the cholesterol content in aortic tissue (reviewed by Lopukhin [7]). The methodology to measure SkTC non-invasively has been standardized and evaluated in studies involving relatively small and well-characterized groups of subjects. This colorimetric technique is reproducible, painless and quick to perform. Studies have shown a correlation between SkTC and abnormal stress tests [3], and between level of SkTC and severity of angiographically defined disease in coronary arteries [4].

The skin contains about 11% of all cholesterol found in the human body [10]. The epidermis has been estimated to contain seven times as much cholesterol by weight as the dermis [11]. Cholesterol is important in maintaining the barrier function of the epidermis. Epidermal cells may also

Table 3
Linear regressions of non-zero agatston coronary artery calcium (CAC) scores (fold change, analyzed on logarithmic scale) on skin/serum lipids/lipoproteins (standard deviation units), MESA skin tissue cholesterol ancillary study (2000–2002)

Serum lipid measure	Caucasian ($n = 74$)		Afr–Amer ($n = 61$)	
	Single predictor β [95% CI]	Multiple predictors β [95% CI]	Single predictor β [95% CI]	Multiple predictors β [95% CI]
TC (/1 S.D.)	1.01 [0.71–1.43]	1.01 [0.67–1.51]	1.07 [0.71–1.61]	1.18 [0.75–1.87]
LDL (/1 S.D.)	0.99[a] [0.70–1.40]	–	1.16[b] [0.75–1.78]	–
HDL (/1 S.D.)	0.88 [0.61–1.25]	0.90 [0.57–1.40]	0.81 [0.52–1.25]	0.71 [0.41–1.24]
TG (/1 S.D.)	1.14 [0.83–1.57]	1.10 [0.74–1.64]	0.97 [0.61–1.55]	0.80 [0.45–1.42]
SkTC (/1 S.D.)	1.04 [0.75–1.40]	1.07 [0.75–1.53]	1.11 [0.72–1.72]	1.06 [0.67–1.68]

β: regression coefficients in terms of units of fold change CAC per 1 standard deviation change in serum lipids or skin tissue cholesterol; for abbreviations and standard deviations see Table 1. All models adjusted for age and sex.

[a] $n = 78$.

[b] $n = 59$; subjects with zero CAC excluded from all analyses.

play a part in removing cholesterol from the body [12]. SkTC is derived from epidermal steroidogenesis and uptake from the circulation. Of the free cholesterol in the skin 39–48% is derived from the circulation [13]. Keratinocytes have been shown to express functional LDL-receptors [14,15] and SRB-1 HDL receptors [16]. Significant positive correlation between epidermal cholesterol and blood plasma apo B has been demonstrated [17]. However, the detailed parallel between epidermal cholesterol metabolism pathways and those in the plaque have not been characterized. Furthermore, non-lipid atherogenic pathways such as systemic inflammation may not have a counterpart with skin cholesterol metabolism.

In this study, SkTC showed a significant association with higher odds of presence of coronary calcium among Caucasian but not African–American subjects. This association was as strong or stronger for SkTC than for serum lipid/lipoprotein measures and was significant even with this modest number of subjects. These data suggest that SkTC may be as predictive or more of the presence of CAC than the traditional serum lipid measures in Caucasians. Though the association did not achieve statistical significance in African–Americans neither did the traditional serum lipoprotein measures.

SkTC was not significantly associated with any of the traditional serum lipid/lipoprotein measures, however, it was associated with hypertension. HDL cholesterol levels indicated greater protection from CAC presence in Caucasians than in African–Americans as for the MESA cohort as a whole (results not shown). However, the extent of protection in Caucasians was much greater in this sample as compared to the whole MESA cohort. Other studies have also shown an inconsistent correlation between cholesterol levels and cardiovascular risk among African–Americans [18–21]. While ethnic differences in the relation between HDL levels and cardiovascular mortality have been shown between Caucasian populations in the US and those in Russia [22,23], no such large-scale study in African–Americans is available. However, serum HDL in African–Americans has been shown to be negatively correlated with measures of socioeconomic status in African–Americans, while the converse is true among Caucasians [24]. This relation may obscure the protective effects of HDL cholesterol in African–Americans, since we did not adjust for socioeconomic status in this study.

The differences in SkTC among racial/ethnic groups seen in this study is not likely due to differences in skin pigmentation. Skin on the palmar surface is poorly pigmented in all groups. Furthermore, there is no difference in the background palmar skin hue (rather than color saturation or value) readings between people with different amounts of body pigmentation [2].

The relationship between hypertension and SkTC is consistent with previous studies [3,4]. The biological basis of this relationship remains to be explored. Since SkTC remains associated with borderline statistical significance with the presence of CAC in Caucasians even after adjustment with hypertensive status, hypertension is not likely to be the only mediator of the relationship between SkTC and CAC.

The lack of correlation between skin and serum cholesterol measures in our study suggests that the amount of cholesterol in the skin may be affected more by the receptor-mediated intake of cholesterol than by the concentration of cholesterol in the serum. This suggests a different biological significance for SkTC from that of serum cholesterol.

All of the cells on the palmar surface are turned over approximately every 28 days [25] and therefore after 2 weeks, enough new cells would be in place to see a change. Thus, this test may serve as a cumulative rather than a “spot” test for the production and deposition of tissue cholesterol.

5. Limitations

This is a cross-sectional study of the relationship between SkTC and measures of subclinical atherosclerosis. As such, associations do not amount to causal relationships. Also, as SkTC is renewed within a few weeks, its lack of association between subclinical atherosclerosis measures in certain groups may not preclude its importance in terms of lifetime exposure. Furthermore, the measures of CAC and IMT are also surrogate measures of cardiovascular risk, and numeric estimates of strength of association may not translate readily to events and mortality.

6. Conclusion

This study suggests that the non-invasive assessment of skin tissue cholesterol content is significantly correlated with the presence of coronary calcification at least among Caucasian subjects. The strengths of the study are that the participants were recruited as part of a population based study with careful collection of blood and standardized IMT and CAC measurements. All IMT and calcium studies were read at central core reading laboratories, and were masked with regards to the SkTC and blood lipid levels. We postulate that the measured SkTC is a sum of the effects of the production, uptake and metabolism of various lipid subfractions. This may explain the independent and strong correlation between SkTC and coronary calcium as compared to individual serum lipids in our sample. The lack of a strong association between total cholesterol, LDL, and TG with CAC and IMT may also be a result of the participants being drawn from a source population with a relatively low prevalence of abnormal lipid levels. Alternatively, or in addition, it may result from insufficient power to detect an association between lipid levels and CAC. The MESA skin tissue cholesterol ancillary study is continuing to recruit participants to confirm these findings.

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Contibution of authors: All authors participated in the analysis, interpretation and critical revision of the manuscript and saw and approved the final version. DV was responsible for the drafting of the manuscript. JD, RK provided statistical expertise, JACL, PO obtained funding.

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