Statin Therapy Has No Significant Effect on Skin Tissue Cholesterol: Results from a Prospective Randomized Trial, Markus Reiter,* Susan Wirth, Ali Pourazim, Mehrdad Baghestanian, Erich Minar, and Robert A. Bucek (Clinic for Internal Medicine II, Department of Angiology, University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria; * author for correspondence: fax 43-1-40400-4665, e-mail markus.reiter@akh-wien.ac.at)

Large-scale clinical trials have conclusively demonstrated that lowering serum cholesterol concentrations with statins slows the progression of atherosclerosis and promotes the regression and stabilization of atherosclerotic plaques, thus substantially reducing the rates of major vascular events among a wide range of patients (1–3).

Skin cholesterol (SkC) has previously been reported to be an independent marker of cardiovascular risk (4–8). The skin test is a quantitative interpretation of extracellular epidermal lipids, which are supplied from dermal blood microcapillaries, the endothelial wall, the uppermost layers of the dermis, and the basement membrane, finally reaching the cells of the basal stratum (9). Thus, lipid-lowering therapy might also have an effect on epidermal lipid content.

Despite the growing interest in this potential new marker of cardiovascular risk, no study has investigated the effects of statins on SkC concentrations. One aim of the present study was to assess the natural midterm course of SkC concentrations prospectively. In addition, we evaluated the potential influence of statin therapy on SkC concentrations as well as potential differences between atorvastatin and simvastatin in a prospective randomized controlled trial.

Consecutive outpatients referred to the department of angiology of a tertiary care university hospital because of suspected vascular disease were eligible for the present prospective trial. Exclusion criteria included (a) current lipid-lowering therapy or lipid-lowering therapy within the last year; (b) age <18 years; (c) pregnancy; (d) psoriasis or eczema on either hand; (e) recent use (within 24 h before testing) of topical medication, as a cream or lotion, on either hand; (f) chronic liver disease or evidence of abnormal liver function (defined as an increase of transaminase activity two times above the upper reference limit of the central laboratory); (g) inflammatory muscle disease and/or an unexplained increase of creatine kinase activity three times above the upper reference limit; (h) conditions that might lead to an incomplete follow-up (i.e., life expectation <6 months); or (i) known incompatibility or allergy against statins. The study protocol was approved by the local ethics committee, and written informed consent was obtained from all patients.

At baseline and 6 weeks and 6 months after start of the study, blood samples were obtained for the determination of total plasma cholesterol (TC), serum LDL- and HDL-cholesterol (LDL-C and HDL-C), triglyceride (TGs), glucose, glycosylated hemoglobin A1c, γ-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, and creatine kinase. SkC concentrations were also measured at baseline and at both follow-up visits by the Cholesterol 1,2,3TM Test (IMI International Medical Innovations Inc.) (6–8). A previous study reported a within-day imprecision (CV) of 11% and a between-day imprecision of 7% for the skin test (6). In the presudy 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, respectively.

The study cohort was then divided into controls (group 1) and patients requiring lipid-lowering therapy (group 2) according to the guidelines of the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), which identifies three categories of risk (2, 10): (a) a LDL-C goal of <1000 mg/L for patients with the highest risk (coronary heart disease or coronary heart disease risk equivalents such as peripheral arterial disease, abdominal aortic aneurysm, symptomatic carotid artery disease, or diabetes mellitus); (b) a LDL-C goal of <1300 mg/L for patients with two or more cardiovascular risk factors; and (c) a LDL-C concentration of <1600 mg/L for patients with no or one risk factor. According to the literature, 40 mg of simvastatin and 20 mg of atorvastatin have similar potential in lowering serum lipids (11). Consequently, group 2 was randomly allocated according to a computer-generated randomization list to group 2a (20 mg of atorvastatin once daily (Sortis®; Pfizer) or group 2b (40 mg of simvastatin once daily (Zocord®; Merck, Sharp & Dohme B.V.).

Between January and April 2003, a total of 129 patients [69 men (54%) and 60 women (46%); mean (SD) age, 63.4 (12.3) years] fulfilled all study criteria and were included in the present trial and assessed at all three visits. Of these, 56 patients had diabetes mellitus (43%), 50 currently smoked cigarettes (38%), and 79 had arterial hypertension (61%). According to the randomization criteria, 44 patients (34%) had serum lipid concentrations.


10. Baghestanian, Erich Minar, and Robert A. Bucek (Clinic for Internal Medicine II, Department of Angiology, University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria; * author for correspondence: fax 43-1-40400-4665, e-mail markus.reiter@akh-wien.ac.at)
within the reference interval and therefore served as the control group (group 1). Increased lipid concentrations required adequate treatment in the remaining 85 patients (66%). These were randomized to either atorvastatin [group 2a; 42 patients (33%)] or simvastatin [group 2b; 43 patients (33%)] treatment (2).

The control and treatment groups differed significantly regarding age [60.1 (13.6) years for group 1 vs 65.1 (11.2) years for group 2; \( P < 0.027 \)], whereas there were no significant differences for gender, body mass index, diabetes mellitus, current cigarette smoking, and arterial hypertension (all \( P > 0.05 \)). The groups differed significantly for the baseline lipids: TC, 1922 (338) vs 2484 (445) mg/L (\( P < 0.001 \)); LDL-C, 1041 (297) vs 1541 (391) mg/L (\( P < 0.001 \)); TGs, 1397 (524) vs 1804 (1034) mg/L (\( P = 0.016 \)). There was no significant difference between groups for HDL-C and SkC (all \( P > 0.05 \)).

The natural course of the investigated variables, as assessed in the control group, revealed a significant decrease (14.4%) in SkC from visit 2 to visit 3 (\( P < 0.001 \)) and a significant increase (6.7%) in LDL (\( P = 0.049 \)). We observed no other significant changes concerning all investigated variables between the baseline assessment and follow-up visits (all \( P > 0.05 \); Table 1 and Fig. 1).

We then analyzed the mean relative changes in the investigated variables at visits 2 and 3. Compared with baseline concentrations, we detected a significant difference in SkC concentrations for group 1 [3.2 (3.3)%] vs group 2 [−4.2 (26.7)%] after 6 weeks (\( P = 0.027 \)) with no additional significant difference after 6 months (\( P > 0.05 \)). As expected, we observed a significant difference between groups 1 and 2 concerning the relative changes in serum lipids. We observed significant decreases in TC, LDL-C, and TG concentrations (each \( P < 0.01 \) after 6 weeks and 6 months), respectively, in the statin groups, whereas there were no relative changes in HDL-C concentrations (\( P > 0.05 \)). There was no statistically significant difference between groups 2a and 2b in lowering SkC (12.1% vs 18.5%; \( P > 0.05 \)) or serum lipid concentrations (all \( P > 0.05 \)).

In review, the present study evaluated for the first time the natural course of SkC by follow-ups for 6 months. Compared with baseline values, SkC increased by 3% after 6 weeks and decreased after 6 months by 12%. These results suggest a significant fluctuation in SkC concentrations, which has also been observed by Zawydiwski et al. (6), who investigated 10 individuals and reported a within-day imprecision of 11% and a between-day imprecision of 7%. A substantial natural variation in SkC concentrations must therefore be considered in interpretation of the results of this novel test (7, 8).

### Table 1. Course of all investigated variables according to treatment groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (group 1)</th>
<th>Atorvastatin (group 2a)</th>
<th>Simvastatin (group 2b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) SkC, arbitrary units</td>
<td>117 (29)</td>
<td>115 (26)</td>
<td>115 (24)</td>
</tr>
<tr>
<td>Baseline</td>
<td>121 (27)</td>
<td>110 (26)</td>
<td>105 (29)</td>
</tr>
<tr>
<td>6 months</td>
<td>103 (23)</td>
<td>101 (15)</td>
<td>94 (16)</td>
</tr>
<tr>
<td>Mean (SD) TC, mg/L</td>
<td>1922 (338)</td>
<td>2490 (411)</td>
<td>2477 (480)</td>
</tr>
<tr>
<td>Baseline</td>
<td>1942 (364)</td>
<td>1802 (536)</td>
<td>1888 (350)</td>
</tr>
<tr>
<td>6 months</td>
<td>2020 (461)</td>
<td>1965 (549)</td>
<td>2031 (457)</td>
</tr>
<tr>
<td>Mean (SD) LDL-C, mg/L</td>
<td>1040 (297)</td>
<td>1552 (332)</td>
<td>1531 (446)</td>
</tr>
<tr>
<td>Baseline</td>
<td>1075 (303)</td>
<td>944 (448)</td>
<td>988 (315)</td>
</tr>
<tr>
<td>6 months</td>
<td>1147 (328)</td>
<td>1136 (459)</td>
<td>1055 (3370)</td>
</tr>
<tr>
<td>Mean (SD) HDL-C, mg/L</td>
<td>602 (186)</td>
<td>558 (188)</td>
<td>624 (165)</td>
</tr>
<tr>
<td>Baseline</td>
<td>575 (196)</td>
<td>536 (157)</td>
<td>608 (170)</td>
</tr>
<tr>
<td>6 months</td>
<td>598 (203)</td>
<td>528 (146)</td>
<td>662 (237)</td>
</tr>
<tr>
<td>Mean (SD) TGs, mg/L</td>
<td>1397 (523)</td>
<td>2005 (1269)</td>
<td>1607 (697)</td>
</tr>
<tr>
<td>Baseline</td>
<td>1503 (714)</td>
<td>1696 (1059)</td>
<td>1459 (699)</td>
</tr>
<tr>
<td>6 months</td>
<td>1629 (854)</td>
<td>1576 (753)</td>
<td>1655 (1323)</td>
</tr>
</tbody>
</table>

* The only significant difference between the baseline values for both treatment groups was for TGs (\( P = 0.048 \)).

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**Fig. 1.** Changes in SkC (A) and TC (B) during the 6-month study period.

- •, group 1 (control group);
- ■, group 2a (atorvastatin);
- ▲, group 2b (simvastatin).
substantial effect on statins of SkC is therefore unlikely or is masked by natural fluctuations in SkC.

The similar changes in SkC concentrations in the control and treatment groups should be analyzed more precisely. Potential explanations include inappropriate drug intake as well as technical failures in test performance. The hypothesis of noncompliance with drug intake in the statin groups can be dismissed in light of the significant decrease in serum lipid concentrations (~30%). Technical failure in the execution of the skin test is also not a probable explanation because the mean (SD) SkC concentrations in our study are comparable to previously published data (6–8). Another potential explanation might be the effect of seasonal variation, but a significant effect seems unlikely, especially if we consider that SkC fluctuations of 11%, even within 1 day, have been reported and that comparable natural fluctuations of 12% were observed in the present study over the total study period of 9 months (6). Nevertheless, based on our present study design, we cannot completely exclude the hypothesis of an additional seasonal influence on SkC.

In summary, SkC concentrations exhibit significant biological fluctuations, and statin therapy leads to comparable decreases in SkC concentrations.

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References

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Comparison of Pre- and Postsurgical Concentrations of Blood HER-2 mRNA and HER-2 Extracellular Domain Reflects HER-2 Status in Early Breast Cancer, Benedetta Salvadori,1 Pamela Pinzani,1 Vito Distanta,3 Donato Casella,3 Simonetta Bianchi,2 Milena Paglierani,2 Vania Vezzi,2 Rainer Neumann,4 Luigi Cataliotti,3 Mario Pazzagl,1 and Claudio Orlando1† (1 Clinical Biochemistry Unit, Department of Clinical Physiopathology, 2 Department of Human Pathology and Oncology, and 3 Department of Surgery, University of Florence, Florence, Italy; 4 Medical Department, Bayer Vital GmbH, Leverkusen, Germany; † address correspondence to this author at: Clinical Biochemistry, Department of Clinical Physiopathology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy; fax 39-055-4271413, e-mail c.orlando@dsc.unifi.it)

The HER-2 gene (HER-2/neu or c-erbB-2) encodes an 185-kDa transmembrane glycoprotein that is a member of the type I family of growth factor receptors. HER-2 is constitutively activated by overexpression and contributes to cell growth, angiogenesis, survival, and metastasis (1). The assessment of HER-2 status in breast carcinomas provides valuable prognostic and predictive information. Immunohistochemistry, fluorescence in situ hybridization, chromosomal in situ hybridization, and quantitative reverse transcription-PCR (RT-PCR) may be used for this purpose. Other approaches have been proposed for the assessment of HER-2 status in peripheral blood, including evaluating either circulating HER-2 extracellular domain (ECD) or nucleated cell-associated HER-2 mRNA. Some studies have indicated that circulating ECD/HER-2 is frequently increased in metastatic disease (2–4). In addition, high concentrations of ECD/HER-2 are associated with cancer aggressiveness (5) and predict response to trastuzumab (6–8) and antiestrogen (4) therapies in advanced breast cancer. Recently, Martin et al. (9), using an array to assess circulating mRNA, pointed out that HER-2 mRNA was generally low in the blood of healthy individuals but was increased in 31% of patients with untreated invasive breast cancer. Almost all of these studies evaluated blood markers in patients with advanced or metastatic breast cancer using a single presurgical sample.

Our study included 40 consecutive patients (median age, 58 years; range, 29–80 years) undergoing surgery for early breast cancer. Patients did not receive any systemic therapy before surgery and provided informed consent for the study. According to tumor size, 28 patients were classified as T1, whereas the remaining 12 were T2 or T3. Twenty-two were node negative, and 35 were positive for estrogen receptors. From each patient, we collected 12 mL of venous blood in EDTA tubes and divided the blood into two 5-mL aliquots. The first was centrifuged, and the plasma was recovered for ECD/HER-2 measurement. The second was used for isolation of nucleated cells by density gradient. Nucleated cell RNA was extracted by TRIzol (Invitrogen), and HER-2 mRNA was measured by real-time RT-PCR. A reference interval was defined in our laboratory using a group of healthy women matched for age with our patients (n = 40). For the breast cancer