Effects of Lovastatin Treatment on Red Blood Cell and Platelet Cation Transport

Alan B. Weder, Carol Serr, Barbara A. Torretti, David R. Bassett, and Andrew J. Zweifler

Hypercholesterolemia frequently accompanies hypertension, and it has been suggested that by affecting membrane lipid composition, hypercholesterolemia may cause or accentuate abnormalities in several red blood cell transports associated with hypertension. Such an effect might obfuscate the relation of membrane markers to hypertension and decrease their usefulness in genetic studies of the heritable basis of hypertension. To determine if changing plasma lipids affects membrane transport, we studied the effects of the cholesterol-lowering agent lovastatin on red blood cell lithium-sodium countertransport and sodium-potassium-chloride cotransport, red blood cell sodium and water content, and platelet amiloride-sensitive volume responsiveness to cytoplasmic acidification, an indirect measure of sodium-proton exchange that has been proposed as a new membrane marker for hypertension. In a 24-week, placebo-controlled, double-blinded, randomized trial, lovastatin significantly lowered total and low density lipoprotein cholesterol and raised high density lipoprotein cholesterol. Red blood cell lithium-sodium countertransport and sodium-potassium-chloride cotransport were not significantly altered. Red blood cell sodium content decreased significantly in the lovastatin-treated group, probably as a result of an increase in red blood cell sodium-potassium pump activity. Platelet amiloride-sensitive responses to cytoplasmic acidification were significantly depressed by lovastatin treatment, suggesting that lowering plasma cholesterol may suppress platelet sodium-proton exchange. It has been hypothesized that the hyperlipidemias frequently observed in essential hypertensive patients may alter membrane lipid composition and affect membrane cation transport activities. Our observations on the effects of lovastatin treatment suggest that the abnormalities in lithium-sodium countertransport and sodium-potassium-chloride cotransport associated with human essential hypertension are unlikely to result from altered membrane cholesterol content or membrane cholesterol/phospholipid ratio. Altered membrane lipid composition may affect the sodium-potassium pump or sodium-proton antiport.

Red blood cell lithium-sodium (RBC Li+-Na+) countertransport is increased in essential hypertension, but the cause of the accelerated flux is unknown. Observations that the \( V_{\text{max}} \) for Li+-Na+ countertransport 1) is increased in some hyperlipidemic patients, 2) correlates significantly with several plasma lipid components, and 3) can be lowered by an exercise program that increases high density lipoprotein (HDL) cholesterol have led to the suggestion that disorders of cholesterol metabolism cause or at least contribute to the increased \( V_{\text{max}} \) for RBC Li+-Na+ countertransport in hypertension. However, it is equally plausible that high total or low HDL cholesterol and hypertension are both features of a common underlying defect, such as that which may cause the syndrome characterized by Williams et al as familial dyslipidemic hypertension. If an increased \( V_{\text{max}} \) for RBC Li+-Na+ countertransport is a marker for a genetic predisposition to familial dyslipidemic hypertension or related hypercholesterolemia syndromes, then increased RBC Li+-Na+ countertransport, hyperlipidemia, and hypertension could all be interrelated, but lipid levels per se would not necessarily contribute to elevated RBC Li+-Na+ countertransport activity.

We measured RBC Li+-Na+ countertransport activity and plasma lipid levels in patients before and after treatment with the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor lovastatin to determine whether alterations in plasma cholesterol affect
RBC Li⁺-Na⁺ countertransport. We also studied the effects of lovastatin treatment on RBC sodium-potassium-chloride (Na⁺-K⁺-Cl⁻) cotransport, another possible marker for hypertension, since previous studies have shown that the Vₘₐₓ for Li⁺-Na⁺ countertransport, the activity of RBC Na⁺-K⁺-Cl⁻ cotransport correlates with plasma lipid levels and in addition, can be affected by alterations in membrane cholesterol both in vitro and in vivo. Finally, we assessed the effect of lovastatin treatment on amiloride-sensitive platelet volume responses to intracellular acidification, an indirect measure of sodium-proton (Na⁺-H⁺) exchange. Although not as well characterized as the RBC transports, amiloride-sensitive platelet swelling is significantly increased in some essential hypertensive patients.

Methods

The present study was approved by the institutional review board for human studies. All participants signed a written consent form after an explanation of the study.

Subjects

Adult nondiabetic male and postmenopausal female volunteers less than 70 years of age and without evidence of coronary artery disease, hypothyroidism, liver disease, or proteinuria were screened in the outpatient Hypertension and Hyperlipidemia Clinics of the University of Michigan for the presence of hyperlipidemia (total cholesterol between 240 and 300 mg%, low density lipoprotein [LDL] cholesterol greater than 160 mg%, and triglycerides less than 350 mg%). Patients receiving lipid-lowering drugs underwent evaluation only after discontinuation of those agents for at least 2 weeks, but no other drug treatments were modified. All patients meeting the above lipid criteria were instructed to continue the AHA Phase I diet through the randomized treatment phase of the study. Qualifying patients were instructed to continue the AHA Phase I diet throughout the study, and dietary instructions were reinforced by the study nurse at intervals. Patients were not permitted to take any additional lipid-lowering drugs during the randomized treatment phase.

Design

The trial was a double-blind, randomized parallel comparison of placebo (administered b.i.d.) and four dosing regimens of lovastatin (placebo qAM/lovastatin 20 mg qPM; placebo qAM/lovastatin 40 mg qPM; lovastatin 20 mg b.i.d.; lovastatin 40 mg b.i.d.).

Blood Pressure, Weight, Height

Blood pressure was measured by the study nurse (C.S.), with the patient in the seated position after 5 minutes quiet rest with the arm elevated to the level of the heart. Heart rate was determined by palpation of the radial pulse. Blood pressures and heart rates reported are the average of two determinations. Weight and height were measured in street clothes without shoes.

Plasma Lipid Methods

Plasma cholesterol and triglycerides were assayed by Smith Kline Bio-Science Laboratories, Van Nuys, Calif. by enzymatic methods. Plasma HDL cholesterol was measured by enzymatic methods in the supernatant fraction after precipitation of the very low density lipoprotein (VLDL) and LDL components by phosphotungstic acid and magnesium chloride. LDL cholesterol was estimated by the Friedewald equation:

\[
\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - \frac{\text{triglycerides}}{5}
\]

Red Blood Cell Transport

RBC content and flux measurements were performed at the end of the run-in period (week 0) and after 24 weeks of treatment (week 24) with either placebo or lovastatin by methods previously described in detail. Briefly, RBC Na⁺ content was determined by atomic absorption (AA) spectrophotometry (Perkin-Elmer 2380 AA Spectrophotometer, Norwalk, Conn.) in trichloroacetic acid–deproteinized hemolysates prepared from washed RBCs, and RBC water content was determined gravimetrically. Measurements of the Vₘₐₓ of RBC Li⁺-Na⁺ countertransport were made using RBC loaded with Li⁺ by incubation in the following (mM): LiCl 150, glucose 10, and Tris-MOPS 10 (pH 7.4 at 37°C) for 3 hours. After loading, RBC were washed free of external Li⁺ in the following cold medium (mM): choline chloride 149, MgCl₂ 1, Tris-MOPS 10 (pH 7.4 at 4°C). Aliquots were suspended in the following Na⁺-rich medium (mM): NaCl 150, glucose 10, ouabain 0.1, Tris-MOPS 10 (pH 7.4 at 37°C) or Na⁺-free medium (mM): choline chloride 149, MgCl₂ 1, glucose 10, ouabain 0.1, Tris-MOPS 10 (pH 7.4 at 37°C). Efflux was initiated by transfer of the cell suspensions to a 37°C shaking water bath. The rate of Li⁺ efflux was calculated from linear regression of extracellular Li⁺ concentration determined by AA spectrophotometry in aliquots removed at 20, 40, and 60 minutes. Li⁺-Na⁺ countertransport was taken as the difference between the Li⁺ efflux rates in Na⁺-rich and Na⁺-free media. The rate coefficient for Li⁺ efflux into the choline medium was calculated as the rate of Li⁺ efflux divided by the initial Li⁺ content of the Li⁺-loaded RBC. We measured the activity of the Na⁺-K⁺-Cl⁻ cotransport system by determining the rate of ouabain-insensitive bumetanide-sensitive Na⁺ efflux into the same choline-based efflux medium used in the Li⁺-Na⁺ countertransport assay above using RBC.
Platelet Transport

Amiloride-sensitive and amiloride-insensitive platelet volume responses to acidification in 140 mM sodium propionate were determined as previously described. Platelet-rich plasma (PRP) was prepared by centrifugation of acid-citrate-dextrose anticoagulated whole blood at 120g for 10 minutes, and platelet volume was measured in a 20 μl aliquot of PRP suspended in an NaCl-based commercially obtained buffer (Isoton II, Coulter Co., Hialeah, Fla.). Signals obtained from a Coulter Model ZM fitted with a 70 μm aperture tube were displayed on a Coulter Channelizer 256, and average platelet volume was determined from the peak of the volume distribution curve. Aliquots of PRP were then suspended in an isosmotic sodium propionate medium (mM): sodium propionate 140, KCl 1, MgCl2 1, CaCl2 1, glucose 10, HEPES 20 (pH=6.7 at 23°C) with and without amiloride (100 μM). Two parameters of amiloride-sensitive responses, the initial rate of volume increase over the first 2 minutes of incubation and the final volume change achieved after 10 minutes, were calculated as previously described in detail. At all time points, amiloride-sensitive platelet volume change was calculated as the difference between volume in the presence and absence of amiloride. The initial rate of amiloride-sensitive volume change is expressed as both an absolute rate (fl/min) and as a fractional rate (amiloride-sensitive volume change divided by platelet volume in the presence of amiloride, as %/min). We also report platelet volume after 10 minutes incubation in sodium propionate plus 100 μM amiloride as a measure of the nonspecific effects of sodium propionate.

Statistical Methods

The effect of lovastatin treatment on plasma lipids was analyzed for significance by analysis of variance according to daily lovastatin dose; for this analysis, the regimens placebo/40 mg lovastatin and 20 mg b.i.d. lovastatin were combined into a single group. When the F test revealed a significant effect, we performed pairwise comparisons by Student's t test to determine significance at each dose level. To determine effects of active treatment on RBC and platelet membrane transports, we combined all active treatment groups and compared transport functions before and after 24 weeks of lovastatin or placebo treatment by paired t test. Relations between variables were examined by bivariate correlation. Significance was accepted at the p=0.05 level. All data are expressed as mean±SEM.

Results

Twenty individuals completed the study: five were given placebo and 15 were treated with lovastatin. As shown in Table 1, there were no significant differences in body weight, blood pressure, or heart rate after 24 weeks of treatment with lovastatin or placebo. Six subjects were treated essential hypertensive patients, three in the placebo group and three in the lovastatin-treated group. Only two patients had alterations in antihypertensive drug treatment during the trial; enalapril was discontinued in one patient in the placebo group and hydrochlorothiazide/amiloride (Moduretic, Merck Sharp & Dohme, West Point, Pa.) was discontinued in a lovastatin-treated subject.

As shown in Table 2, lipids changed substantially during lovastatin treatment: decreases in plasma total (p<0.0001) and LDL (p<0.0001) cholesterol and the rise in plasma HDL cholesterol (p=0.03) were significant by analysis of variance (ANOVA), whereas the fall in plasma triglycerides was not (p=0.10). ANOVA did not reveal a significant effect of drug dose, and paired comparisons (week 0 versus week 24) at each dose level confirmed the significance of the decreases in total and LDL cholesterol on all three active treatment regimens. The increase in HDL cholesterol was significant by paired t test only in the group of patients treated with lovastatin.

### Table 2. Effects of 24 Weeks of Treatment With Lovastatin or Placebo on Plasma Lipids

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 0</th>
<th></th>
<th>Week 24</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>LDL</td>
<td>HDL</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>Placebo (n=5)</td>
<td>230.6±8.0</td>
<td>158.4±11.3</td>
<td>43.4±5.6</td>
<td>143.6±24.1</td>
</tr>
<tr>
<td>Lovastatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mg/day (n=3)</td>
<td>250.7±6.3</td>
<td>181.0±7.2</td>
<td>45.3±4.9</td>
<td>124.0±20.7</td>
</tr>
<tr>
<td>40 mg/day (n=8)</td>
<td>247.3±13.7</td>
<td>179.3±14.4</td>
<td>41.4±2.7</td>
<td>133.0±16.5</td>
</tr>
<tr>
<td>80 mg/day (n=4)</td>
<td>252.3±9.0</td>
<td>182.5±6.7</td>
<td>45.0±3.9</td>
<td>124.0±32.0</td>
</tr>
</tbody>
</table>

*p<0.05; †p<0.01 versus week 0.
TABLE 3. Effects of 24 Weeks of Lovastatin Treatment on Red Blood Cell Cation Transport and Content

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo Week 0</th>
<th>Lovastatin Week 0</th>
<th>Placebo Week 24</th>
<th>Lovastatin Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>15</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Na⁺ content (mmol/l cells)</td>
<td>8.4±1.5</td>
<td>7.5±0.6</td>
<td>7.8±0.4</td>
<td>6.1±0.4*</td>
</tr>
<tr>
<td>H₂O (%)</td>
<td>66.9±0.4</td>
<td>66.4±0.5</td>
<td>66.8±0.3</td>
<td>65.5±0.8</td>
</tr>
<tr>
<td>Li⁺/Na⁺ countercurrent (mmol/l cells/hr)</td>
<td>0.217±0.033</td>
<td>0.207±0.022</td>
<td>0.195±0.028</td>
<td>0.232±0.021</td>
</tr>
<tr>
<td>Rate coefficient passive Li⁺ efflux (min⁻¹)</td>
<td>0.034±0.002</td>
<td>0.037±0.003</td>
<td>0.034±0.004</td>
<td>0.037±0.002</td>
</tr>
<tr>
<td>Bumetanide-sensitive cotransport (mmol/l cells/hr)</td>
<td>0.257±0.076</td>
<td>0.254±0.048</td>
<td>0.176±0.013</td>
<td>0.263±0.055</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.333±0.044</td>
<td>0.283±0.047</td>
<td>0.254±0.039</td>
<td>0.351±0.063</td>
</tr>
</tbody>
</table>

*p<0.05 versus lovastatin week 0.

(40 mg/day). As expected, the falls in total and LDL cholesterol were highly positively correlated (r=0.99, p<0.0001), and the fall in triglycerides was significantly inversely correlated with the rise in HDL cholesterol (r=-0.44, p<0.05).

The effects of placebo and lovastatin treatment on RBC cation metabolism are shown in Table 3. There were no significant effects of placebo treatment on any RBC cation measure. For the lovastatin-treated group, the only significant effect in RBC was a decrease in Na⁺ content; the Vₘₐₓ for Li⁺-Na⁺ countertransport, the rate of Na⁺-K⁺-Cl⁻ cotransport, and the rate coefficient for Li⁺ efflux into a Na⁺-free medium, which measures the sum of the diffusion of Li⁺ through the membrane plus Li⁺-K⁺-Cl⁻ cotransport, were not significantly affected. Treatment with lovastatin did not significantly affect in vitro loading of RBC with Na⁺ for the Na⁺-K⁺-Cl⁻ cotransport measurements [week 0: intracellular sodium concentration ([Na⁺]ᵢ)=44.5±2.9 mmol/l RBC, intracellular potassium concentration ([K⁺]ᵢ)=52.2±4.9 mmol/l RBC; week 24: [Na⁺]ᵢ]=42.0±4.1 mmol/l RBC, [K⁺]ᵢ]=55.0±6.0 mmol/l RBC].

In platelets (Table 4),Lovastatin treatment caused a significant decrease in the initial rate of amiloride-sensitive volume increase caused by propionate acidification and a decrease in the fractional rate of volume change that was of borderline statistical significance (p=0.10). In addition, there was a highly significant effect of lovastatin treatment on the magnitude of the amiloride-sensitive change in platelet volume required for the platelets to reach a new steady state. Median platelet volume in NaCl-based Isoton II did not change significantly with either placebo or lovastatin treatment. Median platelet volume after 10 minutes incubation in 140 mM Na propionate plus 100 μM amiloride was also not significantly affected by placebo or lovastatin treatment. There were significant positive correlations of the final net amiloride-sensitive volume increase with both the initial rate of amiloride-sensitive volume change and the initial fractional volume increase at both week 0 (r=0.41, p<0.05 and r=0.47, p<0.03, respectively) and at week 24 (r=0.59, p<0.01 and r=0.65, p<0.001, respectively); the change in final net volume increase between weeks 0 and 24 was also highly significantly correlated with the changes in the absolute (r=0.71, p<0.001) and fractional (r=0.66, p<0.002) rates. Amiloride-sensitive platelet volume responses to cytoplasmic acidification did not correlate significantly with either RBC Li⁺-Na⁺ countertransport or Na⁺-K⁺-Cl⁻ cotransport rates or with RBC Na⁺ content either at baseline or after treat-

TABLE 4. Effects of 24 Weeks of Lovastatin Treatment on Platelet Size and Amiloride-Sensitive Transport

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo Week 0</th>
<th>Lovastatin Week 0</th>
<th>Placebo Week 24</th>
<th>Lovastatin Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median size (fl):</td>
<td>4.5±0.2</td>
<td>4.7±0.2</td>
<td>4.7±0.3</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>In Isoton II</td>
<td>5.2±0.1</td>
<td>5.4±0.2</td>
<td>5.2±0.2</td>
<td>5.3±0.2</td>
</tr>
<tr>
<td>In 140 mM Na propionate and amiloride</td>
<td>0.94±0.12</td>
<td>1.04±0.05</td>
<td>0.92±0.11</td>
<td>0.93±0.05*</td>
</tr>
<tr>
<td>Amiloride-sensitive responses:</td>
<td>18.2±2.3</td>
<td>19.8±0.9</td>
<td>18.0±2.5</td>
<td>17.7±1.0</td>
</tr>
<tr>
<td>Initial rate (fl/min)</td>
<td>53.8±4.8</td>
<td>53.9±2.0</td>
<td>51.3±4.2</td>
<td>46.8±1.8t</td>
</tr>
</tbody>
</table>

*p<0.05; *p<0.001 versus lovastatin week 0.
ment. Similarly, changes in platelet transport did not correlate with changes in RBC measures.

There were no significant correlations between the magnitude of the changes in total, LDL, or HDL cholesterol or in triglycerides and the changes observed in any of the flux measures.

Discussion

The main new findings in this study are that changing plasma total, LDL, and HDL cholesterol by treatment withLovastatin does not alter the \( V_{\text{max}} \) for RBC Li\(^+\)-Na\(^+\) countertransport or the rate of Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport but does decrease RBC Na\(^+\) content and amiloride-sensitive platelet volume responses to cytoplasmic acidification.

Levy et al.\(^{22}\) demonstrated that increasing RBC membrane cholesterol content in vitro by incubation of RBC with cholesterol hemisuccinate does not affect the \( V_{\text{max}} \) for Li\(^+\)\(-\)Na\(^+\) countertransport even though it decreases passive leak permeability to Li\(^+\). We observed no significant effect of Lovastatin-induced lipid changes on the \( V_{\text{max}} \) for RBC Li\(^+\)\(-\)Na\(^+\) countertransport, nor was the rate coefficient for Li\(^+\) efflux affected, suggesting that any changes induced in membrane composition by the substantial modifications of plasma lipids accompanying Lovastatin treatment are not great enough to affect membrane permeability. The stability of RBC Li\(^+\)\(-\)Na\(^+\) countertransport in the face of such modifications of all plasma lipid components supports the conclusions of Carr et al.\(^{14}\) that hyperlipidemia cannot explain the elevation of the \( V_{\text{max}} \) for RBC Li\(^+\)\(-\)Na\(^+\) countertransport in some essential hypertensive patients and further recommends the use of this cellular marker in studies of the genetic basis of essential hypertension.

Bumetanide-sensitive RBC Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport was also unaffected by the changes in plasma lipids in Lovastatin-treated patients. Although it has been possible to alter RBC Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport by modifying membrane cholesterol content in vitro,\(^{12}\) any changes in membrane cholesterol resulting from Lovastatin treatment must have a smaller, and in the present study a nonsignificant, effect. Because we did not characterize the kinetics of Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport in these patients, it is possible that a subtle functional alteration affecting, for example, the affinity of the cotransporter for internal Na\(^+\), could have been missed.

We did note a significant drop in RBC Na\(^+\) content in Lovastatin-treated patients. Because RBC Na\(^+\) content is directly related to passive membrane cation permeability and inversely related to activity of the Na\(^+\)-K\(^+\) pump,\(^{23}\) it is possible that the fall in RBC Na\(^+\) content we observed is the result of an alteration in either or both parameters. Previous in vitro studies clearly demonstrate that RBC membrane cholesterol enrichment decreases both passive cation leak permeability\(^{22}\) and Na\(^+\), K\(^+\)-ATPase activity,\(^{24}\) and membrane cholesterol depletion stimulates Na\(^+\)-K\(^+\) pump activity.\(^{25}\) Therefore, if the decrease in plasma cholesterol in Lovastatin-treated patients causes a parallel decrease in membrane cholesterol content, both passive permeability and Na\(^+\)-K\(^+\) pump activity could increase, although the degree of cholesterol modification achieved in vitro may be greater than that associated with in vivo Lovastatin treatment. As noted above, the efflux rate coefficient for Li\(^+\) into the choline medium, which includes a flux component determined by passive permeability, did not change as the result of Lovastatin treatment, suggesting that if the fall in RBC Na\(^+\) content accompanying Lovastatin treatment results from changes in membrane lipid composition, it is most likely the result of stimulation of RBC Na\(^+\),K\(^+\)-ATPase. Because neither Na\(^+\),K\(^+\)-ATPase enzyme activity nor ouabain-sensitive fluxes were directly measured in this study, we cannot prove that there is an in vivo effect of Lovastatin treatment on the Na\(^+\) pump, but our results recommend inclusion of measurements of Na\(^+\)-K\(^+\) pump activity in future studies of hypercholesterolemic therapy.

Lovastatin treatment produced a consistent suppression of amiloride-sensitive platelet volume responses to cytoplasmic acidification, which we postulate represents a suppression of platelet Na\(^+\)-H\(^+\) exchange activity. Amiloride-sensitive volume responses to cytoplasmic acidification clearly depend on activation of membrane Na\(^+\)-H\(^+\) exchange,\(^{26}\) and both the absolute and fractional initial rates of amiloride-sensitive volume increase are accepted measures of the initial rate of activation of the Na\(^+\)-H\(^+\) antiporter.\(^{14,15,26}\) In addition, both initial rates of amiloride-sensitive volume increase correlate with net amiloride-sensitive volume increase after 10 minutes, and we and others\(^{15,26}\) have previously suggested that the latter is an additional useful index of Na\(^+\)-H\(^+\) antiporter activation: all three measures are significantly increased in some essential hypertensive patients.\(^{15}\) In the present study, we found good agreement between the effects of Lovastatin treatment on all three measures of amiloride-sensitive transport and no significant effect on basal or amiloride-treated platelet volume. Our interpretation of these findings is that the changes in plasma lipids induced by Lovastatin treatment result in suppression of the activity of the platelet Na\(^+\)-H\(^+\) exchanger.

The technique we used for measuring platelet Na\(^+\)-H\(^+\) exchange, amiloride-sensitive volume change in response to cytoplasmic acidification by sodium propionate, has been criticized because it is indirect and possibly can be influenced by other membrane properties. However, both an increased membrane permeability to Na\(^+\), which might result from a decrease in membrane cholesterol content or a decrease in intracellular Na\(^+\) content such as we observed in RBC, would both be expected to increase amiloride-sensitive platelet volume responses.\(^{27}\) We concede that there may be factors other than the activity of the Na\(^+\)-H\(^+\) antiporter contributing to platelet volume responses to cytoplasmic acidification, but we suggest that our observations are most
consistent with an effect of lovastatin-induced lipid changes on the Na\(^+\)-H\(^+\) antiporter.

Finally, we did not detect a significant correlation between the magnitude of the alterations in any of the plasma lipid fractions and any of the changes in cellular transport or ion content. Although we cannot at this time rule out the possibility that lovastatin had a direct effect on ion transport, we believe that the effects of lovastatin treatment on RBC Na\(^+\) content and platelet amiloride-sensitive volume responses are most likely attributable to the effects of changes in plasma lipids on membrane lipid composition. If the transport changes we describe are due to alterations in membrane cholesterol/phospholipid (C/P) ratio, they might be expected to be more prominent in platelets, which normally have a lower C/P ratio (approximately 0.5\(^2\)) than RBC (approximately 1.0\(^2\)). Future studies should include actual quantitation of changes in RBC and platelet membrane cholesterol content, C/P ratios, and microviscosity during treatment with lipid-altering drugs to help clarify the relation between membrane composition and cation transport. At present, however, based on the evidence in the current study, we cannot support the contention of Bing et al\(^1\) that "Changes in cell membrane lipid composition provide an explanation . . . for the multiple disturbances of monovalent cation fluxes in essential hypertension. . . ."

If the numerous transport abnormalities reported in essential hypertensive patients reflect a common underlying pathophysiological mechanism, our studies suggest that the fundamental defect is not likely to be an alteration of membrane cholesterol or C/P ratio.

In summary, treatment of hypercholesterolemic patients with lovastatin results in significant changes in plasma total, LDL, and HDL cholesterol but has no significant effect on ouabain-insensitive RBC cation transport. The significant decrease in RBC Na\(^+\) content we observed could be related to increased activity of the Na\(^+\) pump in lovastatin-treated patients, but further studies directly measuring pump activity will be necessary to assess that possibility. Lovastatin treatment appears to suppress Na\(^+\)-H\(^+\) exchange in platelets; further direct studies of the effects of hyperlipidemic therapy on Na\(^+\)-H\(^+\) exchange should be undertaken.

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**Key Words** • lithium-sodium countertransport • lipids • sodium-potassium cotransport • sodium-hydrogen exchange • amiloride • cholesterol • membranes • lovastatin
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