Dietary Linoleic Acid Prevents the Development of Deoxycorticosterone Acetate–Salt Hypertension

Jun Kawahara, Hiroshi Sano, Yoshihisa Kubota, Kaoru Hattori, Tetsuo Miki, Hiroshi Suzuki, and Hisashi Fukuzaki

The aim of this study was to elucidate the effect of dietary variations of linoleic acid on the development of deoxycorticosterone acetate (DOCA)–salt hypertension in rats. All rats were divided into three groups and fed one of the following isocaloric diets with 8% NaCl: a high linoleic acid (HLA) (20% sunflower oil), a moderate linoleic acid (5% lard oil + 15% sunflower oil), or a low linoleic acid (DLA) (20% lard oil). After 4 weeks of feeding, we determined intraerythrocyte sodium, potassium, and magnesium concentrations, intra-aortic and lymphocyte magnesium content, and erythrocyte ouabain-sensitive "Na efflux rate constant. Cytoplasmic free calcium concentration of lymphocytes from thymus was also determined with quin-2 as a fluorescent indicator. In the HLA group, the elevation of systolic blood pressure was significantly attenuated, and intraerythrocyte sodium concentration was significantly lower than in the DLA group. There were greater intraerythrocyte potassium and magnesium concentrations, intra-aortic and lymphocyte magnesium contents, and erythrocyte ouabain-sensitive "Na efflux rate constant in the HLA group as compared with other groups. Cytoplasmic free calcium concentration in the HLA group was significantly lower than in other groups. Systolic blood pressure significantly correlated negatively with intraerythrocyte and intra-aortic magnesium concentrations and intraerythrocyte potassium concentration, and correlated positively with intracellular magnesium concentration. Erythrocyte ouabain-sensitive "Na efflux rate constant significantly correlated positively with intraerythrocyte magnesium concentration. These findings suggest that dietary linoleic acid can attenuate the development of DOCA-salt hypertension. It is assumed that the mechanism of its hypotensive effect might be because of a decrease in intracellular sodium and calcium concentration and an increase in intracellular magnesium content, resulting in the elevation of sodium-potassium pump activity. (Hypertension 1990;15(suppl I):I-81–I-87)

Many investigators have proposed the beneficial effect of the dietary polyunsaturated fatty acids (PUFA), especially linoleic acid, on blood pressure in hypertensive subjects and in some forms of experimental hypertension. Although the mechanism is not fully understood, PUFA of plasma membrane has been shown to influence the activity of cation transport such as sodium-potassium adenosine triphosphatase (Na,K)-ATPase, ouabain-insensitive sodium efflux, and calcium-magnesium (Ca,Mg)-ATPase. Cell membrane abnormalities are considered to be implicated in the pathogenesis of primary hypertension and are extensively examined using erythrocytes. Those include altered permeability for univalent cations, changes in calcium-binding ability, and changes in membrane phosphoinositide distribution. We, therefore, postulated that dietary manipulation of lipid composition might induce lipid changes in erythrocyte membrane, resulting in the alteration of membrane ion transport and consequent changes in blood pressure.

The aim of this study was to evaluate the effect of dietary variations of linoleic acid on blood pressure, major cation transport systems of erythrocytes, and cytoplasmic free calcium concentration of thymic lymphocytes in deoxycorticosterone acetate (DOCA)–salt hypertensive rats.

Methods

Six-week-old male Wistar rats were anesthetized with pentobarbital sodium (5 mg/100 g i.p.), and the
left kidneys were removed. After 7 days, they were divided into three groups. These groups were fed one of the following isocaloric diets with 8% NaCl: a high linoleic acid diet (HLA group, n=22) (20% sunflower oil), a moderate linoleic acid diet (MLA group, n=20) (5% lard oil+15% sunflower oil), a low linoleic acid diet (DLA group, n=22) (20% lard oil). All groups of rats were treated for 4 weeks with weekly subcutaneous injections of DOCA, 30 mg/kg body wt in sesame oil. This diet composition was prepared by Oriental Yeast Co. Ltd., Tokyo, Japan, and the fatty acid composition of the oils was determined by gas chromatography (Table 1). Food and water were available ad libitum. Water consumption, food consumption, and weight gain were determined weekly. All rats were housed in individual metabolic cages, and systolic blood pressure (SBP) (mm Hg) was measured once a week by the tail-cuff method using a Narco Biosystems Programmed Electro Sphygmomanometer (PE-3000, Houston, Texas) and 24-hour urinary volume and sodium, potassium, calcium, and magnesium excretion were measured every week. After 4 weeks of treatment, rats were divided into two courses in each group. Rats in the first course (HLA, n=11; MLA, n=10; DLA, n=11) were killed by decapitation to measure 1) intraerythrocyte sodium, potassium, and magnesium concentrations (meq/l, cells), 2) intra-aortic sodium and magnesium concentrations (mM/kg dry wt) after nitric acid digestion, and 3) erythrocyte membrane sodium pump activity. R-Na, R-K, and R-Mg were measured by the method of Kaya et al19 with a slight modification. Erythrocyte membrane sodium pump activity was expressed as erythrocyte ouabain-sensitive ^\text{3}^\text{Na} efflux rate constant (Kos) (/hr) by the method of Walter and Becht20 with a slight modification. Both of these techniques have been described previously.21 Rats in the second course (HLA, n=11; MLA, n=10, DLA, n=11) were killed by decapitation to measure magnesium content (L-Mg) (neq/mg-protein) and cytoplasmic free calcium concentration (L-[Ca^{2+}]) (nM) of lymphocyte from thymus. L-Mg was measured using an atomic absorption spectrophotometer by the method of Girardin and Paunier.22 The protein content was measured using the method of Lowry et al.23 L-[Ca^{2+}] was measured using the

### TABLE 1. Diet Compositions and Dietary Oil Major Fatty Acid Composition

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Diet composition (g/100 g of diet)</th>
<th>14:0</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:1</th>
<th>20:4</th>
<th>20:5</th>
<th>22:1</th>
<th>22:5</th>
<th>22:6</th>
<th>P/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower oil</td>
<td>HLA (n=9) MLA (n=6) DLA (n=6)</td>
<td>6.2</td>
<td>4.5</td>
<td>23.2</td>
<td>64.5</td>
<td>0.3</td>
<td>0.1</td>
<td>0.7</td>
<td>3.4</td>
<td>2.9</td>
<td>0.7</td>
<td>0.3</td>
<td>0.7</td>
<td>0.1</td>
<td>0.06</td>
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<tr>
<td>Lard oil</td>
<td>0 (n=9)</td>
<td>5.0</td>
<td>20.9</td>
<td>1.2</td>
<td>32.7</td>
<td>34.6</td>
<td>0.7</td>
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<tr>
<td>NaCl</td>
<td>20 (n=3)</td>
<td>8.0</td>
<td>8.0</td>
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<tr>
<td>β-Corn starch</td>
<td>15 (n=6)</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
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<tr>
<td>Casein</td>
<td>21 (n=3)</td>
<td>21.0</td>
<td>21.0</td>
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<td>21.0</td>
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<tr>
<td>α-Potato starch</td>
<td>6 (n=11)</td>
<td>6.0</td>
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<td>6.0</td>
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<tr>
<td>Cellulose powder</td>
<td>7 (n=3)</td>
<td>5.0</td>
<td>5.0</td>
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<tr>
<td>Sucrose</td>
<td>2 (n=1)</td>
<td>5.0</td>
<td>5.0</td>
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<td>5.0</td>
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<tr>
<td>Vitamins</td>
<td>2 (n=11)</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
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<td>6.0</td>
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<tr>
<td>Mixed minerals</td>
<td>2 (n=11)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
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</tbody>
</table>

Values are percentages. The (c :d) nomenclature of fatty acid molecules refers to chain length (c), total number of double bonds (d), and (n-x) indicates location of first double bond counting from terminal methyl end. HLA, high linoleic acid diet; MLA, moderate linoleic acid diet; DLA, low linoleic acid diet.

### FIGURE 1. Graphs showing blood pressure (BP) and body weight during the study. HLA, high linoleic acid diet; MLA, moderate linoleic acid diet; DLA, low linoleic acid diet.
TABLE 2. Electrolyte Concentrations in the Plasma and the Aorta After the Study

<table>
<thead>
<tr>
<th>Diet</th>
<th>P-Na (meq/l)</th>
<th>P-Mg (meq/l)</th>
<th>A-Na (mM/kg dry wt)</th>
<th>A-Mg (mM/kg dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA</td>
<td>148.8±3.87</td>
<td>1.29±0.14</td>
<td>9.59±0.61</td>
<td>12.5±1.11</td>
</tr>
<tr>
<td>MLA</td>
<td>147.1±1.7</td>
<td>1.26±0.09</td>
<td>10.13±1.10</td>
<td>11.1±0.80*</td>
</tr>
<tr>
<td>DLA</td>
<td>148.7±2.95</td>
<td>1.34±0.18</td>
<td>9.95±0.50</td>
<td>9.9±0.74*</td>
</tr>
</tbody>
</table>

P-Na, plasma sodium concentration; P-Mg, plasma magnesium concentration; A-Na, intra-aortic sodium concentration; A-Mg, intra-aortic magnesium concentration.

* p<0.005 versus HLA.

HLA, high linoleic acid diet; MLA, moderate linoleic acid diet; DLA, low linoleic acid diet.

TABLE 3. Erythrocyte Ouabain-Sensitive 24Na Efflux Rate Constant and Intraerythrocyte Electrolyte Concentrations After the Study

<table>
<thead>
<tr>
<th>Diet</th>
<th>Kos (hr)</th>
<th>R-Na (meq/l cells)</th>
<th>R-K (meq/l cells)</th>
<th>R-Mg (meq/l cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA</td>
<td>0.775±0.169</td>
<td>7.35±0.58</td>
<td>120.8±2.6</td>
<td>7.19±0.41</td>
</tr>
<tr>
<td>MLA</td>
<td>0.631±0.129</td>
<td>7.95±0.70</td>
<td>115.1±3.1*</td>
<td>8.76±0.24*</td>
</tr>
<tr>
<td>DLA</td>
<td>0.559±0.196†</td>
<td>8.19±0.32†</td>
<td>116.5±2.5*</td>
<td>6.67±0.35*</td>
</tr>
</tbody>
</table>

Kos, erythrocyte ouabain-sensitive 24Na efflux rate constant; R-Na, intraerythrocyte sodium concentration; R-K, intraerythrocyte potassium concentration; R-Mg, intraerythrocyte magnesium concentration.

† p<0.05, †p<0.02, ††p<0.05 versus HLA.

HLA, high linoleic acid diet; MLA, moderate linoleic acid diet; DLA, low linoleic acid diet.

fluence indicator quin-2 by the method of Tsien et al24 with a slight modification.25 The calibration was determined by the method of Hesketh et al.26

The existence of significant difference between groups was examined using analysis of variance, followed by modified t test for individual pair of values. Correlation of the data was sought using a least-squares fit linear regression analysis. Values were expressed as mean±SD. A p value less than 0.05 was considered significant.

Results

SBP during the study is illustrated in Figure 1. SBP in the HLA group was significantly lower than in the DLA and MLA groups at the end of the first week of DOCA treatment. This significant difference continued until the end of the study. There was no significant difference in body weight among the three groups (Figure 1), that is, 242±13 g in the HLA, 239±11 g in the DLA, and 238±13 g in the MLA group at the end of the study. Electrolyte concentrations in the plasma and the aorta of each group after the study are shown in Table 2. There was no significant group difference in plasma sodium, magnesium concentrations, and intra-aortic sodium content. A-Mg was significantly higher in the HLA group than in the other groups. Kos and intraerythrocyte electrolyte concentrations in each group are presented in Table 3. R-Na of the HLA group was lower than that of the other groups. Kos was significantly higher in the HLA group than in the other groups. Kos and intraerythrocyte electrolyte concentrations in each group are presented in Table 3. R-Na of the HLA group was lower than that of the other groups. On the other hand, R-K and R-Mg were significantly higher in the HLA group as compared with the DLA and MLA groups. Kos in the HLA group was higher than in the other groups.

$L-\left[\text{Ca}^{2+}\right]_i$ was 114.3±9.5, the difference between this value and those of the MLA as well as the DLA being significant (Figure 2).

L-Mg was significantly higher in the HLA group (159.2±37.7) than in the MLA and the DLA groups (130.3±15.3 and 130.2±19.7, respectively) (Figure 3) and had no significant correlation with SBP or $L-\left[\text{Ca}^{2+}\right]_i$. Figures 4–6 give the relations among Kos, intraerythrocyte electrolytes, A-Mg, and SBP. As shown in Figures 4 and 5, SBP correlated significantly and negatively with R-Mg, A-Mg, R-K, and Kos. There was also a positive correlation between R-Mg and Kos, and an inverse correlation between R-Na and Kos (Figure 6). The relation between SBP and $L-\left[\text{Ca}^{2+}\right]_i$ is illustrated in Figure 7. SBP correlates significantly and positively with $L-\left[\text{Ca}^{2+}\right]_i$.

Water consumption, urine volume, and food consumption showed no significant difference in any group during the study. Urinary excretion of

![Figure 2. Scatterplot showing cytoplasmic free calcium concentration in thymic lymphocytes (L-\([\text{Ca}^{2+}]_i\)) in each group. HLA, high linoleic acid diet; MLA, moderate linoleic acid diet; DLA, low linoleic acid diet.](http://hyper.ahajournals.org/content/full/1-83/1/fig2)
FIGURE 3. Scatterplot showing lymphocyte magnesium content (L-Mg) in each group. HLA, high linoleic acid diet; MLA, moderate linoleic acid diet; DLA, low linoleic acid diet.

sodium, potassium, calcium, and magnesium are shown in Figure 8. Urinary sodium, potassium, and calcium showed no significant difference in all groups. Urinary magnesium excretion was decreased in the HLA group when compared with the DLA and MLA groups after the third week.

Discussion

In the present study, we observed that the elevation of SBP in DOCA-salt hypertensive rats was significantly attenuated by a high linoleic acid diet.

Earlier studies have shown that PUFA possess significant hypotensive effects both in experimental animals2-8 and in human hypertension occurring with a known high sodium intake.27 This hypotensive action has also been demonstrated in normotensive volunteers.28 Many experiments have revealed that the reduction of blood pressure after dietary linoleic acid intake is mediated by the changes in the prostaglandin metabolism,8,29 a reduced sympathetic nervous activity,3 and the changes of the fluidity in cell membrane.30,31 We demonstrated that sodium-potassium pump activity was elevated greatly in the HLA group. Sodium handling abnormalities have been reported in patients and rats with hereditary forms of arterial hypertension.16,32-34 Interesting negative relations were observed between K0s and both SBP and R-Na, respectively. The mechanisms postulated to explain the hypotensive effect of linoleic acid in salt-loaded hypertension have centered on prostaglandin-mediated mechanisms,35-37 in particular, the possibility of low prostacyclin production from arachidonic acid.38 Although we did not examine the fatty acid composition of erythrocyte cell membrane, it has been reported that membrane lipids are in constant and dynamic equilibrium with plasma lipids.39-41 We, therefore, were interested in the possibility that the mechanism responsible for the effect of linoleic acid might involve modulation of sodium transport by direct modification of the physical characteristics of the cell membrane. Innis and Clandinin42 have shown that dietary fat can directly modulate the physical properties of membranes and associated ATPase activity. Indeed, there is a considerable body of literature reporting such important effects, and this has been reviewed by Wahle.43 Hunt et al,44 in a study of sodium-lithium countertransport, sodium-potassium cotransport, and passive lithium leak, found the passive lithium leak to be most strongly correlated with blood pressure, suggesting a difference in cell membrane "leakiness," as distinct from an alteration in active-transport system. Corrocher et al45 indicated that sodium-lithium countertransport was negatively correlated with the amount of PUFA, whereas it was positively correlated with the oleic-linoleic ratio and the monounsaturated fatty acids-polyunsaturated fatty acids ratio in hyperlipidemic subjects.

We are led to the conclusion that PUFA, especially linoleic acid, can have more beneficial effects on the sodium pump, possibly affecting a lipid
Kawahara et al  The Hypotensive Effect of Dietary Linoleic Acid

The hypotensive effect of dietary linoleic acid 1-85

FIGURE 5. Scatterplots of relations among systolic blood pressure (SBP), intraerythrocyte potassium concentration (R-K), and erythrocyte ouabain-sensitive 2Na efflux rate constant (Kos). HLA, high linoleic acid diet; MLA, moderate linoleic acid diet; DLA, low linoleic acid diet.

microdomain46 that is spatially associated with the pump site, the sodium channel, or both.

In this study, we observed that intracellular magnesium content was elevated in the HLA group and correlated positively with Kos and negatively with SBP. Because magnesium is known to be an important cofactor for activation of (Na,K)-ATPase47 and (Ca,Mg)-ATPase activity,48 increased intracellular magnesium in the HLA group is considered to be partially responsible for elevated Kos and decreased \([Ca^{2+}]_i\), with a consequent decrease in blood pressure. Decreased urinary magnesium excretion seemed to be one of the mechanisms of the increase in intracellular magnesium.

Cytoplasmic calcium in the vascular smooth muscle cell determines the degree of the tension. L-\([Ca^{2+}]_i\) in the MLA and DLA groups was significantly higher than in the HLA group. It can be explained by elevated intracellular sodium concentration in the MLA and DLA groups as compared with the HLA group because intracellular sodium concentration is considered linked with intracellular free calcium concentration by the sodium-calcium exchange system.49,50 Therefore, the hypotensive effect of HLA diets might be due to reduced systemic resistance through decreased intracellular free calcium.

Similar observations have been made regarding control of intracellular sodium content and the ouabain-sensitive sodium efflux rate constant in lymphocytes or leukocytes from normotensive subjects.51 Incubation in serum of hypertensive patients increases both of these parameters to characteristic hypertensive levels.52,53 Among the various circulating compounds capable of modifying both the sodium and calcium transport systems are various lipids that can either act specifically on a given membrane function or induce more widespread modification of the membrane fluidity.

FIGURE 6. Scatterplots showing relations among erythrocyte ouabain-sensitive 2Na efflux rate constant (Kos) and intraerythrocyte sodium and magnesium concentrations (R-Na and R-Mg, respectively). HLA, high linoleic acid diet; MLA, moderate linoleic acid diet; DLA, low linoleic acid diet.
Cholesterol, in particular, is the main rigidifier of natural membranes. An increased membrane cholesterol content enhances membrane microviscosity and decreases sodium and calcium pump activity. Although no data were presented in this study, the dietary linoleic acid significantly decreased cholesterol content of cell membranes in addition to its beneficial effects on blood pressure. Furthermore, the decrease in cholesterol content and the increase in linoleic acid on cell membranes might improve erythrocyte ouabain-sensitive sodium-potassium pump.

Dietary linoleic acid administration played a protective role in DOCA-salt hypertension. It was assumed that the mechanism of its hypotensive effect might be due to an increase in intracellular magnesium content and an elevation of (Na,K)-ATPase activity, which concomitantly caused a decrease in intracellular sodium and free calcium concentration.

References


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KEY WORDS: linoleic acid • DOCA-salt hypertension • sodium-potassium pump • electrolytes • calcium
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