Dietary Calcium and Blood Pressure in Experimental Models of Hypertension
A Review

Daniel C. Hatton, David A. McCarron

Restricted dietary calcium intake has been linked to elevated blood pressure in a number of epidemiologic studies. These findings, in conjunction with indications of calcium deficiency in clinical hypertension, suggest that repletion of dietary calcium may have a beneficial effect on blood pressure. This possibility has been explored extensively in both human and animal models of hypertension. In this article, we review the animal work relating dietary calcium to blood pressure and examine some of the mechanisms that may be responsible. Much of the content is based on published experiments and earlier reviews by our laboratory on this subject.

Background
Calcium Metabolism in Experimental and Essential Hypertension

More than 20 epidemiologic investigations have reported an inverse relation between dietary calcium intake and blood pressure status. The increased risk of elevated blood pressure with lower calcium intake appears to be related to an apparent deficit in calcium metabolism that occurs in a subset of hypertensive individuals. That deficit appears systemically as a small increase in circulating parathyroid hormone (PTH) levels, reduced serum phosphorus, and increased urinary calcium excretion. At the cellular level, the deficit in calcium metabolism is reflected in elevated intracellular free calcium levels, as indicated by studies examining platelets isolated from individuals with essential hypertension.

The alterations in calcium metabolism observed in hypertensive humans are mirrored in experimental models of hypertension. Plasma ionized calcium has been reported to be decreased in the spontaneously hypertensive rat (SHR) relative to the normotensive Wistar-Kyoto (WKY) rat, whereas PTH has been observed to be either elevated or unchanged. End-organ responsiveness to PTH appears to be abnormal based on findings of hypercalcruia, decreased basal and stimulated 1,25-dihydroxyvitamin D₃ [1,25(OH)₂ vitamin D₃] production, and low to normal urinary nephrogenous cyclic AMP in the presence of elevated PTH. At the cellular level, SHR exhibit depressed ATP-dependent calcium uptake capacity in crude microsomal fractions of aorta as well as in more purified sarcolemmal fractions of the mesenteric artery. Therefore, it has been suggested that the vascular myocyte may not be able to maintain normal levels of intracellular calcium or properly remove calcium after activation. It appears that the cell membrane of the SHR as exemplified by platelets and lymphocytes may be hyperpermeable to calcium because both basal and agonist-stimulated calcium uptake have been found to be elevated in the SHR. Consequently, the SHR has abnormally high intracellular free calcium levels, as has been observed in essential hypertensive patients.

The outcome of calcium supplementation in experimental models of hypertension is much more consistent than it is in humans. As shown in Table 1, more than 80 studies have manipulated dietary calcium and measured blood pressure. Most have found an inverse relation between calcium intake and blood pressure. This relation has been found in a variety of genetic and experimental models of hypertension, including SHR; stroke-prone SHR; Dahl salt-sensitive (DS) rats; Lyon hypertensive rats; deoxycorticosterone-saline (DOC-saline) rats; one-kidney, one clip rats; and remnant kidney

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From the Division of Nephrology and Hypertension, Oregon Health Sciences University, Portland.
Reprint requests to Daniel C. Hatton, PhD, Division of Nephrology and Hypertension, PP262, Oregon Health Sciences University, 3314 SW US Veterans Hospital Rd, Portland, OR 97201.
TABLE 1. Studies of Dietary Calcium and Blood Pressure in Experimental Animal Models

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>Calcium Diet</th>
<th>Age of Animal/ Treatment Length, wk*</th>
<th>Result In mm Hg SBP</th>
<th>Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambrozy et al 1991118</td>
<td>Zucker rat</td>
<td>0.5-1.5%</td>
<td>8/4</td>
<td>~↓ 30 in lean, ~↓ 20 in obese, P&lt;.05</td>
<td>Vascular reactivity, VSM Ca2+ efflux</td>
</tr>
<tr>
<td>Anderson et al 1986117</td>
<td>Dog</td>
<td>CaCl2, 115 mEq</td>
<td>2</td>
<td>No effect</td>
<td>Diet-stress interaction</td>
</tr>
<tr>
<td>Anderson et al 1983118</td>
<td>SHR (n=7-8)</td>
<td>0.02-4.0%</td>
<td>16/2</td>
<td>↓ 19 MAP, P&lt;.005</td>
<td>Calcium balance, PTH</td>
</tr>
<tr>
<td>Ando et al 1991119</td>
<td>SD/Ang II/salt</td>
<td>1.17-4.07%</td>
<td>12 days</td>
<td>↓ 35 MAP, P&lt;.01</td>
<td>SNS</td>
</tr>
<tr>
<td>Ayschi 197924</td>
<td>SHR/ WKY</td>
<td>1.2-2.5%</td>
<td>4/14</td>
<td>~↓ 21, P&lt;.05</td>
<td>Metabolic study</td>
</tr>
<tr>
<td>Baks1 198820</td>
<td>SD</td>
<td>0.005/0.17/1.4/2.8%</td>
<td>4/8</td>
<td>~↓ 30, P&lt;.05</td>
<td>Pressor response</td>
</tr>
<tr>
<td>Baks1 198921</td>
<td>SD</td>
<td>0.005/0.17/1.4/2.8%</td>
<td>4/8</td>
<td>↓ 34 MAP, P&lt;.05</td>
<td>CNS NE</td>
</tr>
<tr>
<td>Baks1 198822</td>
<td>SD/PTX</td>
<td>0.005/1.4/2.8%</td>
<td>4/8</td>
<td>↓ 43, P&lt;.05</td>
<td>PTH</td>
</tr>
<tr>
<td>Baks1 et al 1988123</td>
<td>SD</td>
<td>0.005-1.4%</td>
<td>4/8</td>
<td>~↓ 100, P&lt;.01</td>
<td>RAS</td>
</tr>
<tr>
<td>Belizan et al 1981124</td>
<td>Pregnant SD (n=5,6)</td>
<td>0.0-0.6%</td>
<td>20/12</td>
<td>↓ 20, P&lt;.001</td>
<td>Pregnancy-induced hypertension</td>
</tr>
<tr>
<td>Belizan et al 1984125</td>
<td>PTX Wistar (n=9)</td>
<td>0.0-0.6%</td>
<td>8/10</td>
<td>↓ 19, P&lt;.005</td>
<td>PTH</td>
</tr>
<tr>
<td>Blakeborough et al 198025</td>
<td>SHR/WKY</td>
<td>0.06-0.6%</td>
<td>8/10</td>
<td>~↓ 16, P&lt;.05</td>
<td>Intestine and kidney membrane enzyme</td>
</tr>
<tr>
<td>Bogden et al 1981127</td>
<td>Wistar rat</td>
<td>0.02-4.0%</td>
<td>~4/31</td>
<td>~↑ 10 MAP, P&lt;.05</td>
<td>Lead-Ca2+ interaction neoplasia</td>
</tr>
<tr>
<td>Bukoski et al 1989128</td>
<td>SHR/WKY</td>
<td>0.5/1.0/2.0%</td>
<td>6/6 or 12</td>
<td>~↓ 12, P&lt;.05</td>
<td>Vascular contractility</td>
</tr>
<tr>
<td>Bukoski and McCarron 1986129</td>
<td>SHR/WKY</td>
<td>1.0-2.0%</td>
<td>6/8 or 15</td>
<td>~↓ 19, P&lt;.005</td>
<td>Vascular contractility</td>
</tr>
<tr>
<td>DiPette et al 1990130</td>
<td>SD/DOC-salt</td>
<td>0.6-2.5%</td>
<td>~6/8</td>
<td>↓ 14 MAP, P&lt;.05</td>
<td>Calcium-regulating hormones</td>
</tr>
<tr>
<td>Doris 1985131</td>
<td>WKY/saline</td>
<td>1.0-2.5%</td>
<td>~8/26</td>
<td>↓ 10, P&lt;.01</td>
<td>NaCl-Ca2+ interaction</td>
</tr>
<tr>
<td>Doris 1988132</td>
<td>WKY/saline</td>
<td>1.0-2.5%</td>
<td>12/26</td>
<td>~↓ 7, P&lt;.05</td>
<td>NE and PTH</td>
</tr>
<tr>
<td>Evans et al 1990133</td>
<td>SHR (n=9)</td>
<td>0.075/0.5/2.5%</td>
<td>8/23</td>
<td>~↓ 35, P&lt;.001</td>
<td>Mg2+-Ca2+ Interaction</td>
</tr>
<tr>
<td>Fujito et al 1991134</td>
<td>SHR/WKY (n=5)</td>
<td>0.1/0.6/4.0%</td>
<td>6/14</td>
<td>~↓ 12, P&lt;.01</td>
<td>Erythrocyte sodium transport</td>
</tr>
<tr>
<td>Furspan et al 1989136</td>
<td>SHRSP/WKY (n=6)</td>
<td>1.0-2.5%</td>
<td>10/9</td>
<td>↓ 58, P&lt;.05</td>
<td>Membrane stability</td>
</tr>
<tr>
<td>Ganguli et al 1986136</td>
<td>DS rat (n=18)</td>
<td>0.5-3.0%</td>
<td>8/15</td>
<td>↓ 7 MAP, P&lt;.01</td>
<td>CaCO3 vs CaHPO4</td>
</tr>
<tr>
<td>Geiger et al 1986137</td>
<td>SHR/PTX</td>
<td>0.1-3.0%</td>
<td>4/4</td>
<td>↓ 26, P&lt;.05</td>
<td>ANP</td>
</tr>
<tr>
<td>Hano et al 1991138</td>
<td>SHR/WKY (n=10)</td>
<td>1.2% CaCl2 in water</td>
<td>4/3</td>
<td>~↓ 25, P&lt;.01</td>
<td>NE, platelet [Ca2+]</td>
</tr>
<tr>
<td>Hatton et al 1986139</td>
<td>SHR (n=12/21)</td>
<td>0.1-2.0%</td>
<td>Prenatal/4</td>
<td>↓ 30 MAP, P&lt;.01</td>
<td>Early diet experience</td>
</tr>
<tr>
<td>Hatton et al 1987140</td>
<td>SHR/WKY (n=8)</td>
<td>0.1/1.0/2.0%</td>
<td>6/13</td>
<td>~↓ 20, P&lt;.05</td>
<td>Diet-stress interaction</td>
</tr>
<tr>
<td>Hatton et al 1988141</td>
<td>SHR (n=8)</td>
<td>0.1/1.0/2.0%</td>
<td>3/1</td>
<td>↓ 14 MAP, P&lt;.01</td>
<td>Plasma volume, vascular contraction</td>
</tr>
<tr>
<td>Hatton et al 1989142</td>
<td>SHR/WKY (n=8)</td>
<td>0.1/1.0/2.0%</td>
<td>4/12</td>
<td>~↓ 20 MAP, P&lt;.01</td>
<td>SNS</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; VSM, vascular smooth muscle; SHR, spontaneously hypertensive rats; MAP, mean arterial pressure; PTH, parathyroid hormone; SD, Sprague-Dawley rats; Ang II, angiotensin II; SNS, sympathetic nervous system; WKY, Wistar-Kyoto rats; CNS, central nervous system; NE, norepinephrine; PTX, parathyroidectomized; RAS, renin-angiotensin system; DOC, deoxycorticosterone; SHRSP, stroke-prone SHR; DS, Dahl salt-sensitive; ANP, atrial natriuretic peptide; ACI, American Cancer Institute; BP, blood pressure; LVH, left ventricular hypertrophy; PHF, parathyroid hypertensive factor; SHR-S, salt-sensitive SHR; SHR-R, salt-resistant SHR; LHR, Lyon hypertensive rats; 2K1C, two-kidney, one clip hypertensive rats; ADX, adrenalectomized rats; and aldo, aldosterone.

*Unless noted otherwise.

rats. Normotensive rats, such as WKY, Wistar, Fisher 344, and Sprague-Dawley rats, are also responsive to dietary calcium, but the changes in blood pressure are smaller and take prolonged periods of time to develop. The most common method of manipulating dietary calcium has been by altering CaCl2 in dry food. However, CaCl2 in the drinking water has proved to be an effective means of altering blood pressure as well. The
amount of calcium has varied considerably, ranging from 5% to virtually no calcium in the diet. Most studies have used either 0.5% or 1.0% as the normal value for dietary calcium. The smaller value corresponds to the recommended level of dietary calcium for rats based on the American Institute for Nutrition 1976 report.201

<table>
<thead>
<tr>
<th>Study</th>
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<th>Calcium Diet</th>
<th>Age of Animal/Treatment Length, wk*</th>
<th>Result in mm Hg SBP</th>
<th>Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatton et al 1991</td>
<td>SHR (n=8)</td>
<td>0.1-2.0%</td>
<td>3/1</td>
<td>~ ↓ 10 MAP, P&lt;.01</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>Hatton et al 1993</td>
<td>SHR (n=8/7)</td>
<td>0.1-2.0%</td>
<td>3/2</td>
<td>↓ 13 MAP, P&lt;.005</td>
<td>Adrenergic receptors</td>
</tr>
<tr>
<td>Hattori et al 1991</td>
<td>Wistar/DOC-salt (n=9/12)</td>
<td>2.0% CaCl₂ in water</td>
<td>5/4</td>
<td>↓ 56</td>
<td>Endogenous opioids</td>
</tr>
<tr>
<td>Herrmsmeyer et al 1990</td>
<td>SHRSPI</td>
<td>0.2-2.0%</td>
<td>4/8</td>
<td>~ ↓ 25, P&lt;.01</td>
<td>Calcium channels</td>
</tr>
<tr>
<td>Huie et al 1987</td>
<td>Fisher 344, Wistar-Furth, ACI</td>
<td>0.1-1.0%</td>
<td>4/25</td>
<td>↓ 15, P&lt;.05, Fisher 344</td>
<td>Genetic variability</td>
</tr>
<tr>
<td>Jirakulsomchok et al 1990</td>
<td>SHR (n=8/10)</td>
<td>0.1/1.0/2.0%</td>
<td>6/13</td>
<td>~ ↓ 27</td>
<td>Stress-diet interaction</td>
</tr>
<tr>
<td>Jones et al 1990</td>
<td>SHR/DOC-salt/saline (n=7)</td>
<td>0.68-2.0%</td>
<td>7/2.5</td>
<td>↓ 42, P&lt;.05</td>
<td>Natriuresis, diuresis</td>
</tr>
<tr>
<td>Jones and Huibonhoa</td>
<td>SHR (n=9)</td>
<td>1.0-2.5%</td>
<td>~ 4/6</td>
<td>↓ 27, P&lt;.01</td>
<td>Electrolyte interaction</td>
</tr>
<tr>
<td>Kageyama et al 1986</td>
<td>SHR/WKY (n=10)</td>
<td>1.5% CaCl₂ in water</td>
<td>6/3</td>
<td>↓ 26, P&lt;.01</td>
<td>Vascular reactivity</td>
</tr>
<tr>
<td>Kageyama et al 1987</td>
<td>SHR/WKY (n=8)</td>
<td>1.5% CaCl₂ in water</td>
<td>~ 7/3</td>
<td>↓ 53, P&lt;.005</td>
<td>RAS</td>
</tr>
<tr>
<td>Kageyama and Bravo 1987</td>
<td>SHR/WKY (n=8/6)</td>
<td>1% CaCl₂ in water</td>
<td>~ 8/3</td>
<td>~ ↓ 18, P&lt;.01</td>
<td>Vascular reactivity</td>
</tr>
<tr>
<td>Kang et al 1990</td>
<td>Dog/DOC-saline (n=7)</td>
<td>0.4-1.7%</td>
<td>Adult/15 days</td>
<td>↓ 24 MAP, P&lt;.01</td>
<td>Peripheral resistance</td>
</tr>
<tr>
<td>Karanja et al 1987</td>
<td>SHR (n=7)</td>
<td>0.1/0.25/0.5/1.0%</td>
<td>6/14</td>
<td>↓ 13, P&lt;.05</td>
<td>Sodium transport</td>
</tr>
<tr>
<td>Karanja et al 1989</td>
<td>SHR (n=10)</td>
<td>0.25-2.0%</td>
<td>3/26</td>
<td>↓ 16, P&lt;.01</td>
<td>Fat/calcium</td>
</tr>
<tr>
<td>Kohno et al 1989</td>
<td>SHR/WKY (n=30)</td>
<td>0.4-2.9%</td>
<td>10/3</td>
<td>↓ 22, P&lt;.05</td>
<td>ANP</td>
</tr>
<tr>
<td>Koido and Tuan 1988</td>
<td>Chick embryo (n=8-9)</td>
<td>0%</td>
<td>Prenatal</td>
<td>↓ 3 MAP, P&lt;.05</td>
<td>Adrenergic regulation</td>
</tr>
<tr>
<td>Kurtz and Morris 1986</td>
<td>DOC-saline (n=7-9)</td>
<td>0.5-4.0%</td>
<td>4/9</td>
<td>↓ 26 MAP</td>
<td>DOC-calcium interaction</td>
</tr>
<tr>
<td>Lau et al 1984</td>
<td>SHR/WKY (multiple groups)</td>
<td>0.22/1.2/4.3%</td>
<td>Multiple experiments</td>
<td>~ ↓ 15, P&lt;.05</td>
<td>PTX, PO₄, volume, hypercalcemia</td>
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<tr>
<td>Lau et al 1986</td>
<td>SHR (n=10)</td>
<td>0.87-2.0%</td>
<td>3/12</td>
<td>↓ 16, P&lt;.05</td>
<td>Na⁺/Ca²⁺ interaction, RAS</td>
</tr>
<tr>
<td>Lau et al 1986</td>
<td>SHR/WKY (n=11;14)</td>
<td>0.35/0.87/1.35%</td>
<td>3-15/5-11</td>
<td>No effect</td>
<td>Calcium balance</td>
</tr>
<tr>
<td>Lee et al 1984</td>
<td>Turkey (n=10)</td>
<td>0.98/1.99%</td>
<td>33/15</td>
<td>↓ 29 MAP, P&lt;.005</td>
<td>LVH, stress</td>
</tr>
<tr>
<td>Lewanczuk et al 1990</td>
<td>SHR (n=10)</td>
<td>0.02/0.6/2.0%</td>
<td>4/8</td>
<td>↓ 33 MAP, P&lt;.01</td>
<td>PHF</td>
</tr>
<tr>
<td>Luft et al 1988</td>
<td>SHRSP, WKY (n=20)</td>
<td>0.25-4.0%</td>
<td>6/16</td>
<td>↓ 24, P&lt;.05, no effect of higher Mg</td>
<td>Mg homeostasis, catecholamines</td>
</tr>
<tr>
<td>Mangiarua et al 1990</td>
<td>SHR/WKY (n=12-60)</td>
<td>0.02/1.0/4.0%</td>
<td>5/8</td>
<td>↓ 24 MAP, P&lt;.05</td>
<td>Hypertensive factor</td>
</tr>
<tr>
<td>McCarron et al 1981</td>
<td>SHR/WKY (n=12)</td>
<td>0.25/0.5/4.0%</td>
<td>10/38</td>
<td>~ ↓ 30, P&lt;.001</td>
<td>Calcium metabolism</td>
</tr>
<tr>
<td>McCarron 1982</td>
<td>WKY (n=12)</td>
<td>0.25/0.5/4.0%</td>
<td>8-10/24-26</td>
<td>↓ 14, P&lt;.001</td>
<td>Calcium balance</td>
</tr>
<tr>
<td>Study</td>
<td>Model</td>
<td>Calcium Diet</td>
<td>Age of Animal/Treatment Length, wk*</td>
<td>Result In mm Hg SBP</td>
<td>Focus</td>
</tr>
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<td>-----------------------------</td>
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<td>--------------------------------------------</td>
</tr>
<tr>
<td>McCarron et al 1985</td>
<td>SHR/WKY (n=7)</td>
<td>0.1/1.0/2.0%</td>
<td>6/14</td>
<td>↓ 56, P&lt;.01</td>
<td>Na-Ca2+ interaction, Ca2+ flux</td>
</tr>
<tr>
<td>Muntzel et al 1989</td>
<td>SHR (n=10-14)</td>
<td>0.1/1.0/2.0%</td>
<td></td>
<td>~ ↓ 14 MAP, P&lt;.01</td>
<td>Early exposure</td>
</tr>
<tr>
<td>Oparil et al 1991</td>
<td>SHR-S, SHR-R, WKY (n=10-12)</td>
<td>0.66-2.0%</td>
<td>8/2</td>
<td>~ ↓ 14 MAP, P&lt;.01</td>
<td>NaCl, SNS</td>
</tr>
<tr>
<td>Oshima et al 1992</td>
<td>SHRSP (n=15)</td>
<td>0.2-2.0%</td>
<td>3/6</td>
<td>~ ↓ 35, P&lt;.001</td>
<td>Cellular calcium</td>
</tr>
<tr>
<td>Panmali et al 1990</td>
<td>Wistar/reduced renal mass (n=21)</td>
<td>-1.0-3.0%</td>
<td>-10/5</td>
<td>↓ 40, P&lt;.05</td>
<td>Electrolyte metabolism</td>
</tr>
<tr>
<td>Pang et al 1992</td>
<td>SHR (n=12)</td>
<td>0.2/0.4/0.8%</td>
<td>12/8</td>
<td>↓ 12 MAP, P&lt;.05</td>
<td>Calcium antagonists</td>
</tr>
<tr>
<td>Pernot et al 1978/79</td>
<td>SD DOC-saline (n=7-12)</td>
<td>3.5% CaCl2 in water</td>
<td>-6/10</td>
<td>↓ 25, P&lt;.01</td>
<td>PTH</td>
</tr>
<tr>
<td>Pernot et al 1985/79</td>
<td>SD DOC-saline SHR, LHR</td>
<td>DOC=1.3% CaCl2 in water; SHR=0.3-1.2%, LHR=0.6-2.5%</td>
<td>6/16 DOC, 6/44 SHR, 6/22 LHR</td>
<td>~ ↓ 30 DOC, SHR, LHR</td>
<td></td>
</tr>
<tr>
<td>Pernot et al 1990/77</td>
<td>LHR (n=7-12)</td>
<td>0.03/0.6/2.5%</td>
<td>4/23</td>
<td>~ ↓ 22, P&lt;.01</td>
<td>Vascular reactivity</td>
</tr>
<tr>
<td>Peuler et al 1987/78</td>
<td>DS rat (n=10-11)</td>
<td>0.4-4.0%</td>
<td>4/6-7</td>
<td>~ ↓ 30 MAP, P&lt;.05</td>
<td>SNS</td>
</tr>
<tr>
<td>Peuler and Mark 1989/79</td>
<td>DS rat (n=10-13)</td>
<td>0.4-2.0%</td>
<td>6/6</td>
<td>~ ↑ 25, P&lt;.05</td>
<td>Calcium BP, SNS</td>
</tr>
<tr>
<td>Peuler 1991/80</td>
<td>SHRSP/DS rat (n=8-13)</td>
<td>0.4-2.0%</td>
<td>4-6/6-22</td>
<td>DS=↑ 17 MAP SHRSP=↑ 38 MAP, P&lt;.05</td>
<td>Divergent effects calcium on BP</td>
</tr>
<tr>
<td>Peuler and Schelper 1992</td>
<td>SHRSP (n=11-13)</td>
<td>0.4-2.0%</td>
<td>4 Weeks to death</td>
<td>~ ↓ 45 MAP, P&lt;.05</td>
<td>Stroke</td>
</tr>
<tr>
<td>Porst et al 1990/182</td>
<td>SHR DOC-saline (n=12)</td>
<td>1.5% CaCl2 in water</td>
<td>8/9</td>
<td>↓ 24 MAP, P&lt;.05</td>
<td>Vascular reactivity, Ca2+-ATPase</td>
</tr>
<tr>
<td>Porst et al 1992/182</td>
<td>SHR/WKY (n=16/9)</td>
<td>1.1-3.1%</td>
<td>8/12</td>
<td>↓ 20 MAP, P&lt;.0001</td>
<td>Vascular relaxation, platelet [Ca2+], Na,K-ATPase</td>
</tr>
<tr>
<td>Ports 1992/184</td>
<td>SHR/WKY (n=16/9)</td>
<td>1.1-2.1%</td>
<td>8/12</td>
<td>↓ 18 MAP, P&lt;.0001</td>
<td>Vascular relaxation, platelet [Ca2+]</td>
</tr>
<tr>
<td>Resnick et al 1986/185</td>
<td>Wistar DOC-saline/2K1C (n=10)</td>
<td>0.2-1.8%</td>
<td>-6/4</td>
<td>↓ 16 DOC, ↑ 21 K1C, P&lt;.05</td>
<td>RAS</td>
</tr>
<tr>
<td>Resnick et al 1986/186</td>
<td>Wistar DOC-saline/2K1C</td>
<td>1.2-1.8%</td>
<td>-6/4</td>
<td>↓ 23 DOC, ↑ 18 K1C, P&lt;.05</td>
<td>RAS</td>
</tr>
<tr>
<td>Saito et al 1991/61</td>
<td>WKY DOC-saline (n=8-30)</td>
<td>1.0-4.0% CaCl2</td>
<td>6/4</td>
<td>↓ 55, P&lt;.01</td>
<td>Electrolyte balance</td>
</tr>
<tr>
<td>Schieffer et al 1984/47</td>
<td>SHR (n=7-9)</td>
<td>0.0/0.3/1.2%</td>
<td>5/39</td>
<td>~ ↓ 18, P&lt;.05</td>
<td>BP</td>
</tr>
<tr>
<td>Scroggin et al 1991/84</td>
<td>SHR (n=8-9)</td>
<td>0.2-2.0%</td>
<td>4/8</td>
<td>~ ↓ 18, P&lt;.05</td>
<td>Na-Ca2+, BP reactivity</td>
</tr>
<tr>
<td>Scroggin et al 1991/189</td>
<td>SHR (n=8-9)</td>
<td>0.2-2.0%</td>
<td>4/8</td>
<td>↓ 14, P&lt;.05</td>
<td>Stress</td>
</tr>
<tr>
<td>Samafuko and Morris 1991/180</td>
<td>SHR/ADX-aldo (n=8-10/4-5)</td>
<td>0.5-2.5%</td>
<td>3/17 SHR, 6/2 SHR/ADX</td>
<td>↓ 20 SHR, ↑ 70 SHR/ADX, P&lt;.01</td>
<td>Calcium/aldosterone</td>
</tr>
<tr>
<td>Stern et al 1984/50</td>
<td>SHR/WKY (n=10)</td>
<td>0.4-2.8%</td>
<td>6/4</td>
<td>No effect</td>
<td>Calcium metabolism</td>
</tr>
<tr>
<td>Stern et al 1987/83</td>
<td>SHR (n=18)</td>
<td>0.4-2.5%</td>
<td>10/4</td>
<td>No effect</td>
<td>Vascular reactivity</td>
</tr>
</tbody>
</table>
However, 1.0% is often considered the normal level because it is the amount contained in major brands of rat chow.

Based on the available literature, it appears that studies which have used fairly substantial differences in calcium intake (5- to 10-fold) between treatment groups have most consistently reported significant differences in blood pressure. Using five diets that ranged between 0.1% and 2.0% calcium, Karanja et al.\textsuperscript{157} reported a nonlinear inverse relation between the calcium content of the diet and blood pressure in SHR maintained on a concurrent high sodium chloride diet.\textsuperscript{172-199-200} It is of interest to note that animals on enriched calcium diets eat as much as 30% more but weigh less than animals on normal calcium diets.\textsuperscript{203}

**Proposed Mechanisms for the Antihypertensive Actions of Calcium**

A multitude of potential mechanisms may explain the effect of dietary calcium on blood pressure. As dietary calcium changes, so do PTH, calcitriol, calcitonin, calcium gene–related peptide (CGRP), atrial natriuretic peptide (ANP), renin-angiotensin system activity, sympathetic nervous system activity, metabolism of other electrolytes, calcium binding proteins, intracellular free calcium levels, membrane-bound calcium, and electrolyte fluxes. Any one or combination of these changes could be responsible for altering blood pressure. In the following sections, several mechanisms that have been postulated to mediate the effects of dietary calcium on blood pressure will be explored. The proposed mechanisms are summarized in Table 2.

**Vascular Smooth Muscle**

Calcium plays a key role in vascular smooth muscle function. Calcium influx through receptor- and voltage-operated calcium channels is thought to initiate vascular contraction, and the fall in the intracellular free calcium concentration is thought to result in relaxation or vasodilation. Therefore, how the vascular smooth muscle cell (VSMC) handles calcium is critical to vascular tone and blood pressure. Anything that causes a perturbation in the processes that control cellular regulation of calcium would very likely change vascular tone.

In the following sections, the effects of dietary calcium on VSMC regulation of calcium will be considered. In some instances, the effects on vascular smooth muscle are estimated from the effect of dietary calcium on platelets or other cell types. Although there are impor-
TABLE 2. Proposed Mechanisms of Action of Supplemental Dietary Calcium on Blood Pressure in Experimental Models of Hypertension

<table>
<thead>
<tr>
<th>Site of Action</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular</td>
<td>† Membrane stabilization</td>
</tr>
<tr>
<td></td>
<td>† Na,K-ATPase</td>
</tr>
<tr>
<td></td>
<td>† Ca-ATPase</td>
</tr>
<tr>
<td></td>
<td>† Calmodulin</td>
</tr>
<tr>
<td></td>
<td>▼ Intracellular calcium</td>
</tr>
<tr>
<td>Vascular</td>
<td>▼ Contractility</td>
</tr>
<tr>
<td></td>
<td>† Relaxation</td>
</tr>
<tr>
<td>Calcium-regulating hormones</td>
<td>▼ PTH</td>
</tr>
<tr>
<td></td>
<td>▼ 1,25(OH)(_2) vitamin D(_3)</td>
</tr>
<tr>
<td></td>
<td>† Calcitonin</td>
</tr>
<tr>
<td>Calcium-sensitive hormones</td>
<td>▼ Parathyroid hypertensive factor</td>
</tr>
<tr>
<td></td>
<td>▼ Atrial natriuretic peptide</td>
</tr>
<tr>
<td></td>
<td>▼ Renin-angiotensin system</td>
</tr>
<tr>
<td></td>
<td>† CGRP</td>
</tr>
<tr>
<td>Sympathetic nervous system</td>
<td>▼ CNS outflow</td>
</tr>
<tr>
<td></td>
<td>▼ Circulating catecholamines</td>
</tr>
<tr>
<td></td>
<td>▼ α-Adrenergic receptor-mediated responses</td>
</tr>
<tr>
<td>Renal</td>
<td>† Prostaglandins</td>
</tr>
<tr>
<td></td>
<td>† Natriuresis</td>
</tr>
</tbody>
</table>

† Indicates increase in; ▼ decrease in; PTH, parathyroid hormone; CGRP, calcitonin gene-related peptide; and CNS, central nervous system.

Membrane Stabilization

Based on numerous reports of abnormalities in cell membrane transport systems associated with hypertension, Bohr and colleagues have hypothesized that there is a defect in the lipid bilayer of vascular smooth muscle membrane in hypertensive individuals. This defect results in decreased membrane fluidity and enhanced excitability as a consequence of increased Ca\(^{2+}\) flux across the cell membrane. Bohr and coworkers have provided persuasive evidence that increased extracellular calcium causes a reduction in vascular contractility through a process of membrane stabilization. Increased binding of calcium to the plasma membrane decreases membrane permeability to both monovalent and divalent cations, resulting in diminished vascular contractility.

Membrane stabilization is an attractive hypothesis for explaining the effects of dietary calcium, but most studies of membrane stabilization have been done in vitro using extracellular calcium levels that may not correspond to the magnitude of change that might be expected from manipulations of dietary calcium. To evaluate the effect of dietary calcium on cellular function, Furspan and Bohr fed stroke-prone SHR and WKY rats either 1% or 2.5% calcium diets for 10 weeks. They then measured blood pressure, serum ionized calcium, and lymphocyte intracellular sodium, potassium, and calcium, as well as net passive fluxes of sodium and potassium. They found that supplemental calcium reduced blood pressure, serum ionized calcium, and lymphocyte intracellular sodium, potassium, and calcium, as well as net passive fluxes of sodium and potassium. They found that supplemental calcium reduced blood pressure, increased serum ionized calcium, reduced net potassium efflux, and reduced free ionized calcium within the lymphocyte. The changes were attributed to increased extracellular calcium and resultant membrane stabilization in the calcium-supplemented stroke-prone SHR.

Na,K-ATPase

In a series of studies, Porsti et al. reported that increasing dietary calcium lowers blood pressure and improves vascular relaxation in the SHR. They found that supplemental calcium normalized DOC-induced
elevations of blood pressure as well as the increased vascular contractility to norepinephrine that is characteristic of vessels from DOC-treated animals. Dietary calcium did not alter contractility to norepinephrine in SHR not treated with DOC, suggesting that the change in maximal contractility resulted from a calcium-induced modulation of the DOC treatment. On the other hand, supplemental calcium did enhance relaxation to sodium nitroprusside and acetylcholine in both the DOC-calcium group and the calcium-supplemented control SHR. The authors suggest that the improved relaxation may have been a consequence of increased Na,K-ATPase activity. In a previous report, they noted that potassium-induced relaxation of mesenteric arterial rings was augmented by calcium supplementation in SHR. Because ouabain was able to prevent the relaxation, the difference between diet groups was attributed to increased Na,K-ATPase activity. This outcome is consistent with the notion of a reciprocal relation between intracellular calcium and Na,K-ATPase activity; as intracellular calcium declines, Na,K-ATPase activity increases.

The results from Porsti et al. are commensurate with other findings showing the maximal contractility to norepinephrine in intact vessels is not altered by supplemental dietary calcium. Aorta, mesenteric, and tail arteries from animals fed high calcium diets have not shown diminished reactivity to norepinephrine or potassium chloride (KCl).* If anything, there may be increased contractility. Ambrozy et al. found increased sensitivity to phenylephrine in Zucker obese and lean rats fed high calcium diets, whereas Pernot et al. found an increase in maximal contractility to norepinephrine in Lyon hypertensive rats on high calcium diets.

Despite the in vitro results, blood pressure reactivity in vivo has been reported to be reduced to norepinephrine in animals fed supplemental calcium. Several investigators have reported diminished pressor responses to norepinephrine in both hypertensive and normotensive rats fed high calcium diets. These results would not appear to be a consequence of diminished vascular contractility given the results from isolated vessels cited above. However, the in vivo results may be related to circulating factors that modify the response to norepinephrine or to difficulties related to vascular relaxation.

Ca\(^{2+}\)-ATPase

Just as Furspan and Bohr observed lower levels of intracellular free calcium in lymphocytes from animals on high calcium diets, Porsti et al. reported lower levels of intracellular free calcium in platelets isolated from animals maintained on supplemental calcium diets. These favorable changes in intracellular calcium regulation may be related to increased Ca\(^{2+}\)-ATPase activity. Porsti et al. and Wuorela et al. observed an increase in the maximal velocity of calcium transport by “inside-out” red blood cell vesicles from SHR on calcium-supplemented diets. The greater velocity of calcium transport is indicative of increased Ca\(^{2+}\)-ATPase activity. Wuorela et al. also reported that nitroprusside-induced relaxation of norepinephrine-contracted mesenteric arterial rings was enhanced in calcium-supplemented animals, perhaps as a consequence of an increased ability to extrude calcium from the cell.

Calmodulin

Using duodenal enterocytes, Roullet et al. have observed that calmodulin levels are lower in SHR than WKY rats and that increasing dietary calcium eliminates that difference. Subsequent work indicated that dietary calcium can actually upregulate calmodulin levels (C.M. Roullet, personal communication, November 1993). Correction of a defect in calmodulin activity by calcium could provide a mechanism whereby a multitude of molecular and cellular processes might be modified. Calmodulin plays a pivotal role in intracellular calcium regulation and could be responsible for diet-induced variations in Ca\(^{2+}\)-ATPase.

Calcium-Regulating Hormones

Until recently, calcium-regulating hormones were rarely considered outside of their traditional role in calcium homeostasis. Calcium-regulating hormones exert tight control over serum free ionized calcium levels (for review, see Reference 215). Reductions in serum ionized calcium provoke release of PTH from the parathyroid gland. PTH in turn stimulates the production of calcitriol [1,25(OH)\(_2\) vitamin D\(_3\)], the most active metabolite of vitamin D, in the proximal convoluted tubule cell. Calcitriol enters the circulation to reach its target tissues, the principal targets being the renal and intestinal epithelia where 1,25(OH)\(_2\) vitamin D\(_3\) increases the synthesis of the calcium-binding proteins calbindin and integral membrane calcium-binding protein. An increase in these calcium-binding proteins is reflected by greater intestinal absorption and renal reabsorption of calcium. When serum free ionized calcium levels are too high, calcitonin is released from the thyroid gland. This compound acts to lower serum free ionized calcium by inhibiting osteoclast activity and preventing mobilization of calcium from bone.

It has become increasingly clear that PTH, calcitriol, and perhaps calcitonin possess vasoactive properties and thus may play a role in blood pressure regulation. Furthermore, other vasoactive peptides and hormones have been identified that are responsive to dietary calcium such as CGRP and parathyroid hypertensive factor. Because these compounds are responsive to changes in calcium intake, it follows that variations in calcium-regulating hormones may account for calcium-induced alterations in blood pressure.

Parathyroid hormone. Hyperparathyroidism is associated with elevated blood pressure in humans, and PTH is elevated in many essential hypertensive patients. These observations suggest that elevated PTH may in some way be responsible for the high blood pressure. If so, dietary calcium may lower blood pressure by suppressing PTH. However, such an effect would be paradoxical because PTH is a potent vasodilator. Intravenous injections of PTH result in a prompt, dose-dependent fall in blood pressure. Nevertheless, parathyroidectomy has been consistently shown to lower blood pressure in animal models of hypertension. Furthermore, transplantation of parathyroid glands between WKY rats and SHR results in higher
blood pressure in the WKY rat and lower blood pressure in the SHR. 226,229,230

The resolution of the paradox may be related to a number of factors. First, it should be noted that at physiological concentrations in blood, PTH does not appear to be vasoactive. 231-233 Thus, the influence of PTH on blood pressure may be independent of its vasodilating effects. Second, prolonged exposure to low calcium diets, which elevates circulating PTH levels, 50 diminishes the magnitude of the blood pressure decline after an acute injection of PTH. 114 The potential development of tolerance to the vasodilator effects of PTH may be associated with a simultaneous enhancement in response to other vasoactive drugs. This situation would be analogous to the effect of alcohol exposure on arterial pressure. Acutely, alcohol is a vasodilator, but with continued exposure it is associated with hypertension. Several studies have shown that as the vasodilator effects of alcohol decline, there is an increase in intracellular free calcium in VSMCs that may favor enhanced responses to other agonists (for review, see Reference 234).

PTH may act as a calcium ionophore to promote increased intracellular calcium levels in VSMCs and thereby increase vascular tone. The possibility of PTH as an ionophore in vascular smooth muscle is suggested by findings that low concentrations of PTH induce a calcium influx into isolated cardiac cells 235 as well as osteoblasts. 236 The relation between PTH and platelet intracellular calcium has been examined in humans and in rats. Oshima et al 237 could find no effect of parathyroidectomy on platelet free intracellular calcium in SHR. In humans, there is little intercorrelation between intracellular free calcium and PTH activity. 238 Thus, the evidence does not favor the ionophoric effect of PTH in hypertension.

It is possible that PTH has central nervous system effects that promote an elevation of blood pressure. In a brief article, Delbarre et al 239 reported that intracerebral ventricular administration of PTH resulted in significantly elevated blood pressure in the SHR. Although this study is apparently the only one of its kind, there are other indications that PTH has central nervous system effects. Geiger et al 240 reported altered cyclic AMP in various brain regions in the rat that were attributable to variations in PTH. More recently, Islam et al 241 found evidence that PTH diminished synaptic somal phospholipid content in uremic rats. In a subsequent study, Smogorzewski et al 242 reported that PTH was responsible for a higher calcium content in rat brain synaptosomes as well as reduced Na,K-ATPase activity. PTH also modified epinephrine content, uptake, and release.

An additional explanation for the PTH paradox is the putative parathyroid hypertensive factor. Lewanzuk et al 243 have reported the presence of a circulating hypertensive factor that is present in both SHR and human plasma. 244 When injected into normotensive rats for bioassay, the hypertensive factor stimulates vascular smooth muscle calcium uptake and causes an elevation in blood pressure with a time lag of about 2 hours. Parathyroidectomy eliminates the circulating factor, suggesting that the parathyroid gland is the source. 229 If so, this would shift focus away from PTH to the parathyroid gland itself and in so doing would reconcile the disparate blood pressure results that have previously been ascribed to PTH. Pertinent to this review, the purported factor is responsive to variations in dietary calcium. Increased dietary calcium reduces circulating hypertensive factor, whereas restricted calcium increases it. 247

Calcitriol. As mentioned previously, low calcium diets provoke an increase in the synthesis and release of 1,25(OH)2 vitamin D3, (see Reference 215), which itself may mediate an increase in blood pressure. Receptors for 1,25(OH)2 vitamin D3 have now been demonstrated in vascular tissue. 246,247 Calcitriol has been shown to stimulate Ca2+-ATPase in vascular smooth muscle, suggesting that 1,25(OH)2 vitamin D3 may play a role in regulating cellular calcium metabolism. 248,249 A direct effect of calcitriol on VSMC intracellular free Ca2+ has been reported for calcitriol in intact mesenteric resistance arteries as well as isolated VSMCs. Bukoski et al 250 and Xue et al 251 have shown that short-term incubation of intact mesenteric resistance vessels with 1,25(OH)2 vitamin D3 increases the Ca2+ transient induced by norepinephrine in the SHR. No effects were seen in the WKY rat. Similar effects are observed after systemic injections of calcitriol. Bukoski et al 252,253 observed that both acute injections and 3-day administration of the vitamin at physiological levels significantly enhanced maximal force generation of isolated vessels from both hypertensive and normotensive animals. These findings suggest direct effects of 1,25(OH)2 vitamin D3 on Ca2+ metabolism of intact resistance vessels. They further suggest that the observed response to calcitriol differs depending on whether the vascular tissue is isolated from the normal or hypertensive animal.

Despite evidence of direct effects on vascular smooth muscle, observations of elevated blood pressure as a consequence of exposure to 1,25(OH)2 vitamin D3 have been inconsistent. The animals that Bukoski et al 252 examined for vascular contractility did not have elevated blood pressure as a consequence of exposure to calcitriol. Likewise, Hatton et al 253 found evidence of enhanced vascular contractility in vessels from animals given 1,25(OH)2 vitamin D3 injections for 7 consecutive days, but there were no changes in blood pressure. Using a higher concentration of 1,25(OH)2 vitamin D3, Shimosawa et al 254 did observe a potentiation of pressor responses to both norepinephrine and angiotensin II in animals treated with calcitriol or the noncalcemic analogue 22-oxacalcitriol for 14 days. Most recently, Bukoski and Xue 255 found evidence that daily injections of 1,25(OH)2 vitamin D3 over a 28-day period caused sustained elevations in blood pressure in normotensive Wistar rats. However, chronic administration of 1,25(OH)2 vitamin D3 in SHR produced only a transient elevation in blood pressure after 5 weeks of treatment, suggesting a modest effect of the hormone on blood pressure. 256 As reported in previous studies, vascular contractility was significantly enhanced in the SHR. Thus, considering the direct effects on the vasculature, the observation of potentiated pressor responses, and recent reports of elevated blood pressure, 1,25(OH)2 vitamin D3 should be considered as an agent that has the potential to alter blood pressure. Further
Calcitonin. In general, the effects of calcitonin on calcium regulation are opposite to those of PTH and 1,25(OH)₂ vitamin D₃. Although supplemental dietary calcium can be expected to elevate calcitonin levels, calcitonin has not been routinely measured in studies of the effect of dietary calcium manipulations on blood pressure. Nevertheless, there is evidence that calcitonin may have vasoactive properties.

There have been conflicting reports on the relation of calcitonin to blood pressure. Bindels et al.⁴⁹ found significantly elevated calcitonin in young SHR, along with reduced serum ionized calcium and elevated 1,25(OH)₂ vitamin D₃. Presumably, the disparate results between elevated calcitonin in the presence of reduced serum ionized calcium were related to phosphate metabolism. These findings suggest that calcitonin is associated with elevated blood pressure. In fact, Clementi et al.²⁵⁷ reported elevated blood pressure after intracerebroventricular administration. Wegener and McCarron²⁵⁸ were unable to detect any effect of calcitonin on blood pressure through intravenous administration.

Additional research will be needed to assess the effects of calcitonin on blood pressure and its relation to dietary calcium. Calcitonin may well have an influence on blood pressure via the central nervous system. Although calcitonin is a thyroid hormone, a number of calcitonin receptors in the brain²⁶⁰ mediate a variety of effects, including anorexia,²⁶¹ analgesia,²⁶² and altered pituitary release of hormones.²⁶² However, at the present time there is insufficient evidence to evaluate the possibility that calcitonin modulates blood pressure via the central nervous system.

Calcitonin gene-related peptide. In contrast to calcitonin, CGRP is known to be vasoactive. This 37-amino acid neuropeptide results from alternative processing of the calcitonin gene. It is widely distributed in the central and peripheral nervous systems²⁶³ and is one of the most potent vasodilators yet discovered. Intravenous infusion of CGRP produces marked hypotension through a reduction in total peripheral resistance.²⁶⁴ Central nervous system administration of CGRP produces variable effects depending on the dose and site of administration. For example, injection of 0.2 pmol CGRP into the nucleus tractus solitarius produced a depressor response, whereas 2 pmol resulted in a pressor response.²⁶⁶ Likewise, injections into the central nucleus of the amygdala resulted in a pressor response,²⁶⁸ but intrathecal injections caused a reduction in blood pressure.²⁶⁸

Of particular interest are observations that levels of CGRP in spinal cord vary with dietary calcium. DiPette et al.²⁶⁹ demonstrated that low levels of dietary calcium

work is needed to understand the cellular events underlying the vascular smooth muscle response to calcitriol.

There are consistent reports of altered sympathetic nervous system activity associated with variations in dietary calcium. Winternitz et al.²⁷¹,²⁷² and Oparil et al.²⁷³ have reported that increased sodium chloride causes an elevation of blood pressure in salt-sensitive SHR. The increased blood pressure is associated with elevated circulating norepinephrine and reduced hypothalamic norepinephrine.²⁷⁴ Provision of supplemental calcium reverses the increased blood pressure induced by sodium chloride while normalizing circulating and central nervous system catecholamines.²⁷⁵,²⁷⁶,²⁷⁷ Pueler et al.²⁷⁸ reported that 4.8% calcium diets reduce blood pressure in DS rats by attenuating sympathetic nervous system outflow. Interestingly, 2% calcium diets aggravated hypertension in this strain²⁷⁹ while reducing blood pressure in the stroke-prone SHR.³⁸⁰

There are several possible mechanisms through which calcium may modify the sodium chloride effect on sympathetic nervous system outflow. One possibility is that the electrolytes may interact within the central nervous system. Infusion of sodium chloride into the cerebral ventricles²⁸² or nucleus tractus solitarius²⁸³ has been shown to cause an elevation of blood pressure. In contrast, intracerebroventricular calcium causes a reduction in blood pressure.²⁷⁷ Therefore, it appears that both calcium and sodium chloride are reciprocally vasoactive in the central nervous system. There is evidence that manipulations of dietary calcium result in altered levels of central nervous system calcium. Long-term alterations in dietary calcium have been shown to change cerebrospinal fluid and brain levels of calcium in rats. Harris et al.²⁸⁴ found the reduction in calcium levels to be greatest in the brain stem (24%) after 4 weeks of restricted dietary calcium (0.02%), with the soluble and microsomal fractions of calcium selectively reduced. Likewise, Tai et al.²⁸⁵ found that calcium fluxes into brain and cerebrospinal fluid were linearly related to plasma ionized calcium. Thus, it appears that although the brain is protected from acute changes in serum ionized calcium by low cerebrovascular permeability to calcium, long-term changes in dietary calcium can alter brain levels of calcium, albeit to a lesser extent than the changes in serum calcium.²⁸⁰

The interaction between sodium and calcium may occur at the receptor level. Gavras²⁸¹ has hypothesized that sodium may directly influence neural activity and cause increased sympathetic nervous system outflow by attenuating the affinity of α₂-adrenergic receptors for their agonists. Because activation of hypothalamic α₂ receptors causes inhibition of sympathetic outflow, reduced affinity would result in disinhibition of the sympathetic system. In contrast to sodium, divalent cations such as calcium increase the affinity of the receptors for the ligand.²⁸²
Whether changes in central nervous system calcium levels are responsible for alterations in central catecholamine levels remains to be demonstrated. Nevertheless, reductions in dietary calcium comparable to those used to modify central nervous system calcium levels are reported to alter central nervous system norepinephrine levels. Baksi et al. reported that 8 weeks of a 0.005% calcium diet reduced hypothalamic norepinephrine and dopamine content in Sprague-Dawley rats. Restricted dietary calcium has also been reported to alter peripheral catecholamine levels. Baksi and Hughes and Hagi-hara et al. reported altered adrenal catecholamine levels with reduced calcium diets, and Luft et al. observed reduced epinephrine levels and epinephrine responses to stress in stroke-prone SHR on high calcium diets. Scrogin et al. also observed diminished circulating epinephrine in calcium-supplemented SHR. Felicetta et al. observed an increased urinary dopamine-norepinephrine excretion ratio during a high calcium/high sodium chloride diet. Other investigators have reported no difference in circulating catecholamines as a consequence of altered calcium diets. 

Responses to norepinephrine have been reported to be potentiated by restricted calcium diets and dampened by high calcium diets. Hatton et al. reported diminished pressor responses to exogenous norepinephrine in SHR on high calcium diets. The reduced pressor response did not occur in response to angiotensin II, suggesting that it was not due to a generalized change in vascular responsiveness. Doris, Kageyama et al., and Luft et al. observed reduced epinephrine levels and epinephrine responses to stress in stroke-prone SHR on high calcium diets. Scrogin et al. also observed diminished circulating epinephrine in calcium-supplemented SHR. Felicetta et al. observed an increased urinary dopamine-norepinephrine excretion ratio during a high calcium/high sodium chloride diet. Other investigators have reported no difference in circulating catecholamines as a consequence of altered calcium diets.

The reduced responsiveness to norepinephrine may be related to altered adrenergic receptor activity. Hatton et al. demonstrated that dietary calcium specifically modulates the $\alpha_1$-adrenergic receptor. Blockade of $\alpha_1$-adrenergic receptors with phentolamine or prazosin eliminated the difference in blood pressure that prevailed on animals on high and low calcium diets. Blockade of $\beta_2$, $\beta_3$, or $\beta_3$-adrenergic receptors had no such effect. Likewise, pharmacologic reduction of blood pressure with CGRP, sodium nitroprusside, or the converting enzyme inhibitor captopril had no differential effect on blood pressure. The results of this study strongly suggest that dietary calcium modifies the $\alpha_1$-adrenergic receptor. The nature of the effect remains to be determined, but preliminary data indicate that there may be a difference in receptor expression because binding to tritiated prazosin was greater in kidneys from animals fed low calcium diets.

**Electrolyte Interactions**

A change in the level of one dietary electrolyte can have extensive ramifications on other dietary electrolytes. Increased dietary calcium, for example, can increase sodium excretion, reduce magnesium absorption, and reduce circulating phosphorus levels. Likewise, increasing sodium chloride in the diet causes calcium wasting, but if sodium levels are altered independently of chloride, calcium is unaffected. Consequently, when manipulation of a dietary electrolyte is shown to have an effect on blood pressure, the possibility exists that the blood pressure effect is due to reciprocal changes in other electrolytes.

**Phosphate depletion.** Early in the study of the effect of dietary calcium on blood pressure, Lau and coworkers proposed that calcium-induced phosphate depletion accounted for the blood pressure reduction. However, rather than a positive relation between phosphate and blood pressure as predicted by Lau et al., Bindels et al. demonstrated that supplementing the SHR diet with phosphate results in a blood pressure reduction during mid-adolescence. To specifically determine the role of phosphorus in calcium-induced reductions in blood pressure, Tamura varied both calcium and phosphate and found that calcium lowered blood pressure regardless of serum phosphate levels. Thus, it would appear that the effect of calcium on blood pressure is relatively independent of phosphate.

**Magnesium.** Dietary calcium intake can have a pronounced effect on magnesium metabolism. With increased dietary calcium intake, serum magnesium levels decline. Furthermore, observations of increased intracellular calcium with low calcium diets suggest that intracellular magnesium may be reduced. Because of the interactive effects of dietary calcium and magnesium, it has been suggested that some of the effects of calcium on blood pressure may be mediated through alterations in magnesium. Indeed, Tamura found a significant positive correlation of serum magnesium levels with systolic blood pressure across groups receiving different levels of dietary calcium. However, in a factorial study of calcium and magnesium, Evans et al. found no effect of magnesium on blood pressure and no interactive effects of the two cations on blood pressure.

**Sodium chloride/calcium interactions.** Numerous studies have found that calcium attenuates sodium chloride-dependent, low-renin hypertension. Calcium lowers blood pressure in several salt-sensitive models of hypertension, including DOC-saline rats, reduced renal mass/saline rats, DS rats, salt-sensitive SHR, and adrenalectomy/aldosterone-induced hypertension. The mechanisms through which calcium attenuates salt-induced forms of hypertension have not been identified, but numerous candidates have been suggested.

**Calcium-induced natriuresis.** Increasing dietary calcium facilitates natriuresis in a number of ways. Increased circulating ANP, reduced sympathetic nervous system outflow, reduced $\alpha_1$-adrenergic receptor activity, reduced angiotensin II receptor expression, reduced circulating PTH, and direct effects of calcium on sodium excretion at the proximal tubule may all play a role. The most familiar natriuretic effect of calcium is the interaction that occurs at the renal level. Increasing calcium facilitates natriuresis and diuresis in part by inhibiting sodium reabsorption in the proximal tubule. Conversely, reducing serum ionized calcium levels by acute volume expansion retards natriuresis and diuresis. This occurs, at least in part, as a consequence of increased circulating PTH acting on the tubules to alter production of prostaglandins, with a resultant reduction in sodium excretion as well as direct effects of calcium on prostaglandin production. Less well known are the effects of increasing dietary calcium on other systems that promote natriuresis. Geiger et al. and Kohno et al. have reported increased circulating ANP after supplemental calcium diets. Such an increase in ANP...
would promote natriuresis as well as cause peripheral vasodilation and reduced blood pressure. Levi and Henrich recently reported that high calcium diets reduced angiotensin II binding in renal brush-border membranes and decreased brush-border membrane Na-H antiport activity in SHR. Angiotensin II increases proximal tubular sodium transport by increasing Na-H antiport activity. Decreased activity in this system would enhance sodium excretion.

The effect of calcium on sympathetic nervous system activity would also facilitate sodium excretion. The reduction in sodium chloride-induced increases in sympathetic nervous system outflow that have been reported can be expected to result in increased sodium excretion, as would the diminished \( \alpha \)-adrenergic activity during high calcium diets, as reported by Hatton et al.

Given the multiple mechanisms through which increased dietary calcium facilitates sodium excretion, it is not surprising that increasing the proportion of calcium in the diet results in natriuresis. In some models of hypertension, natriuresis might be an important component of the antihypertensive effect of dietary calcium. This would apply regardless of how sodium chloride might provoke hypertension, because removal of sodium chloride would remove the initial stimulus for elevated blood pressure.

Not all evidence favors a natriuretic explanation for the pressure blood effects of dietary calcium. In SHR that are not salt sensitive, increasing dietary sodium chloride does not alter blood pressure. However, increasing dietary calcium does lower blood pressure. Studies from our laboratory and that of Hamet et al. have demonstrated that the antihypertensive action of calcium may actually be enhanced by simultaneous sodium chloride supplementation. This interactive effect of these nutrients is not likely to be related to natriuresis because, as mentioned above, manipulation of sodium chloride alone does not alter blood pressure in these animals.

Thus, it may be necessary to distinguish between hypertensive models when assessing the interactive effects of dietary calcium and sodium chloride. For models in which calcium prevents a sodium chloride-induced elevation in blood pressure, such as in DOC-salt hypertension, natriuresis might be suspected. Otherwise, natriuresis seems less likely. Even when natriuresis is an unlikely cause for the blood pressure-lowering effect of dietary calcium, as in the SHR, sodium chloride and calcium may interact to potentiate the antihypertensive effects of supplemental calcium.

**Sodium chloride-induced calciuresis.** Just as calcium promotes natriuresis, sodium chloride promotes calciuresis. Consequently, as sodium chloride is increased in the diet, more calcium is excreted. Resnick and colleagues hypothesize that this dietary sodium chloride-induced calciuresis results in reduced serum ionized calcium and increased circulating levels of calcium-regulating hormones including calcitriol, PTH, and/or parathyroid hypertensive factor. In turn, these hormones stimulate cellular calcium uptake and disrupt cellular electrolyte balance, resulting in elevated blood pressure. According to this hypothesis, calcium supplementation increases available calcium, prevents the fall in ionized calcium that provokes the increase in calcium-regulating hormones, and reverses the salt-induced hypertension.

Favoring this hypothesis are indications that increased dietary sodium chloride causes renal wasting of calcium and stimulates calcitriol and PTH production. Elevated calcitriol and PTH have been found in low-renin human hypertension as well as in experimental forms of hypertension. Kotchen et al. reported that after 5 days of a high salt diet, young DS rats exhibited increased calcium excretion in both urine and feces, whereas ionized calcium was reduced and calcitriol and PTH were elevated relative to Dahl salt-resistant rats. The differences in calcium excretion occurred with no changes in blood pressure. The excessive calcium excretion could be avoided by sodium loading with anions other than chloride, suggesting that the combination of sodium and chloride is critically important to the calciuresis that occurs. It is notable that high sodium diets that do not contain chloride also do not cause hypertension in the DS rat. Similarly, Kurtz and Morris found increased calcium excretion in DOC-saline hypertension when the animals were fed sodium chloride. However, if an equimolar amount of sodium bicarbonate was substituted for sodium chloride, the increases in both calcium excretion and blood pressure were reversed.

To test the hypothesis that sodium chloride causes hypertension as a consequence of alterations in calcium-regulating hormones, Resnick et al. fed supplemental dietary calcium to low-renin, DOC-salt hypertensive rats and to renin-dependent Goldblatt hypertensive rats. Calcium excretion was substantially greater in the DOC-salt rats relative to the two-kidney, one clip Goldblatt rats. Modest increases in dietary calcium (1.2% versus 1.8%) caused a reduction of blood pressure in the DOC-salt animal but resulted in an increase in blood pressure in the Goldblatt model of hypertension.

Although these results are dramatic, other investigators have found that supplemental dietary calcium reduces renovascular hypertension. Kageyama et al. reduced blood pressure in two-kidney, one clip Goldblatt hypertensive rats by providing supplemental calcium (1.5%) in the drinking water. The reduction in blood pressure was accompanied by a suppression of plasma renin and aldosterone as well as diminished pressor responses to norepinephrine, leading the authors to conclude that the reduction in blood pressure may have been a consequence of reduced renin-angiotensin system activity.

**Nonvascular Mechanisms**

Nonvascular mechanisms must also be considered in explaining the role of dietary calcium in blood pressure regulation. Tordoff and colleagues have provided important new insights as to potential nonvascular mechanisms. Their studies in three genetically distinct animal models indicate that dietary calcium regulates sodium chloride appetite or intake. Low dietary calcium stimulates sodium chloride intake, and higher intakes of calcium suppress sodium chloride appetite. Thus, while volume may not be changed at higher calcium intakes,
exposure to sodium chloride is reduced with potentially important effects on sodium-regulating hormones such as renin, aldosterone, and angiotensin II.

Change in blood viscosity is another nonvascular mechanism potentially affected by dietary calcium. Hatton et al.143 have measured changes in hematocrit that occur with calcium supplementation as an indirect assessment of viscosity. They found that there is an inverse relation between calcium intake and hematocrit. However, the changes in blood pressure observed with the higher calcium intake were independent of changes in hematocrit.

Hamet and Tremblay299 have linked correction of the abnormal HSP70 gene expression of experimental hypertension to increased dietary calcium intake. The HSP70 gene, which exists in the major histocompatibility complex and segregates closely with hypertension, has its expression corrected (suppressed) by dietary calcium.299

Summary

More than 80 studies have reported lowered blood pressure after dietary calcium enrichment in experimental models of hypertension. The evidence presented here suggests that dietary calcium may act concurrently through a number of physiological mechanisms to influence blood pressure. The importance of any given mechanism may vary depending on the experimental model under consideration.

Supplemental dietary calcium is associated with reduced membrane permeability, increased Ca2+-ATPase and Na,K-ATPase, and reduced intracellular calcium. These results suggest that supplemental calcium may limit calcium influx into the cell and improve the ability of the VSMC to extrude calcium. This could be a direct effect of calcium on the VSMC or an indirect effect mediated hormonally.

The calcium-regulating hormones have all been found to have vasoactive properties and therefore may influence blood pressure. Furthermore, CGRP and the proposed parathyroid hypertensive factor are both vasoactive substances that are responsive to dietary calcium. Therefore, diet-induced variations in calcium-regulating hormones may influence blood pressure.

Modulation of the sympathetic nervous system is another important way that dietary calcium can influence blood pressure. There is evidence of altered noradrenaline levels in the hypothalamus as a consequence of manipulations of dietary calcium as well as changes in central sympathetic nervous system outflow. Dietary calcium has also been shown to specifically modify α1-adrenergic receptor activity in the periphery.

In some experimental models of hypertension, dietary calcium may alter blood pressure by changing the metabolism of other electrolytes. For example, the ability of calcium to prevent sodium chloride–induced elevations in blood pressure may be attributed to natriuresis. However, natriuresis does not account for all of the interactive effects of calcium and sodium chloride on blood pressure. Sodium chloride–induced hypertension may be due in part to calcium wasting and subsequent elevation of calcium-regulating hormones. Chloride is an important mediator of this effect because it appears that sodium does not cause calcium wasting when it is not combined with chloride.

More attention to the central nervous system effects of dietary calcium is needed. Not only can calcium itself influence neural function, but many of the calcium-regulating hormones appear to affect the central nervous system. The influence of calcium and calcium-regulating hormones on central nervous system activity may have important implications for blood pressure regulation and also may extend to other aspects of physiology and behavior.

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D C Hatton and D A McCarron

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