Enhanced Vascular Reactivity and Ca$^{2+}$ Entry With Low-Salt Diet

Effect of Obesity

Raouf A. Khalil, Janice K. Crews, Joan F. Carroll, John E. Hall

Abstract—Salt moderation is often recommended as a nonpharmacological therapy for hypertension, particularly in overweight individuals; however, the effects of low dietary salt on the Ca$^{2+}$-dependent mechanisms of vasoconstriction are unclear. The purpose of this study was to investigate the effect of low salt diet on vascular reactivity and Ca$^{2+}$ mobilization mechanisms and the modulation of these effects with obesity. Active stress and $^{45}$Ca$^{2+}$ influx were measured in deendothelialized aortic strips isolated from lean (3.74 kg) and obese (5.51 kg) female rabbits on a normal (0.75%) or low (0.23%) salt (sodium chloride) diet for 18 weeks. Both phenylephrine (Phe, $10^{-5}$ mol/L) and membrane depolarization by 96 mmol/L KCl caused extracellular Ca$^{2+}$-dependent increases in active stress and $^{45}$Ca$^{2+}$ influx. In lean rabbits, the Phe- and KCl-induced stress and Ca$^{2+}$ influx were significantly greater with the low-salt versus the normal-salt diet. The Phe-induced Ca$^{2+}$ influx–stress relationship was significantly greater than that induced by KCl with low-salt diet. In obese rabbits on a normal-salt diet, the Phe- and KCl-induced stress and Ca$^{2+}$ influx were significantly less than that in lean rabbits but the Ca$^{2+}$ influx-stress relationship was not significantly altered. Feeding the obese rabbits a low-salt diet was associated not only with significant increases in Phe- and KCl-induced active stress and Ca$^{2+}$ influx but also with significant enhancement in the Ca$^{2+}$ influx-stress relationship. In Ca$^{2+}$-free (2 mmol/L EGTA) Krebs solution, stimulation of intracellular Ca$^{2+}$ release by Phe or caffeine (25 mmol/L) caused a transient contraction that was not significantly different in all groups of rabbits. Thus, with normal salt intake, obesity is associated with a reduction in Ca$^{2+}$ entry and vascular reactivity. Low-salt diet is associated with an increase in Ca$^{2+}$ entry and vascular reactivity in both obese and lean rabbits. The enhancement of the Ca$^{2+}$ influx-stress relationship with low-salt diet, particularly in the obese rabbits, suggests activation of other contractile mechanisms in addition to Ca$^{2+}$ entry. (Hypertension. 1999;34[part 2]:882-888.)

Key Words: diet ■ resistance, vascular ■ calcium ■ muscle, smooth, vascular ■ contraction

Obesity is recognized as a serious health problem in the United States, where an estimated 33% of the adult population is markedly overweight.1 In some segments of the population, such as elderly African-American women, the prevalence of obesity is as high as 75%.2 Obesity is associated with several systemic and regional alterations in hemodynamics, increased intravascular volume, increased cardiac output, decreased overall peripheral vascular resistance, and, commonly, hypertension.3,5

Studies in the obese dog model have shown that obesity is associated with marked sodium retention and expansion of extracellular fluid volume.6,6 Also, as in other forms of hypertension, obesity hypertension is invariably associated with a shift in the pressure-natriuresis curve toward higher pressure.6 Rocchini and coworkers3 also have observed a reduction in the slope of pressure-natriuresis in obese adolescents and found that this change is reversible with weight loss. The decreased slope of pressure natriuresis caused blood pressure to be highly salt sensitive, with low salt intake greatly ameliorating the hypertension in obese subjects. However, studies in abdominally obese adults <45 years of age have shown that in a large number of the subjects studied blood pressure was not sensitive to salt intake.7 Also, Granger and Nakamura8 have shown that obesity hypertension in dogs is not salt-sensitive and is characterized by a parallel shift, rather than a decreased slope, in pressure natriuresis. The cause of these discrepancies is unclear but could be related, as could several other factors, to the possibility that the predicted beneficial effects of low-salt diet in reducing the extracellular fluid volume and the intravascular volume in the obese individuals are counterbalanced by additional effects of low-salt diet on other control mechanisms, such as the peripheral vascular resistance. However, the changes in vascular reactivity and the Ca$^{2+}$ mobilization mechanisms in
obesity, particularly with low-salt diet, have not been clearly elucidated.

The purpose of the present study was to determine whether low-sodium diet, in the presence or absence of obesity, is associated with increases in vascular reactivity and the Ca\(^{2+}\) mobilization mechanisms of vascular smooth muscle contraction (ie, Ca\(^{2+}\) release from the intracellular stores and Ca\(^{2+}\) entry from the extracellular space). Therefore, active stress and Ca\(^{2+}\) entry were measured and the Ca\(^{2+}\) entry-active stress relationships were constructed and compared in vascular strips isolated from lean and obese rabbits on normal- or low-sodium diets. The obese rabbit model was used because it has been shown to mimic many of the cardiovascular changes noted in obesity-associated hypertension in humans and other animal models.\(^5\)\(^6\)

**Methods**

**Animals**

Female New Zealand White rabbits (15 to 17 weeks of age, 3 to 3.25 kg; Myrtle’s Rabbitry, Thompson Station, Tenn) were housed in the animal facility of the University of Mississippi Medical Center and fed 100 to 120 g/d standard rabbit chow. After a 2-week acclimation period, the rabbits were divided into 4 groups: lean on normal-salt, lean on low-salt, obese on normal-salt, and obese on low-salt diet. The normal-salt groups were fed a diet that contained 0.75% sodium chloride, and the low-salt groups were fed a diet that contained 0.23% sodium chloride. The lean groups were fed standard rabbit chow. The obese groups were given a high-fat diet ad libitum that consisted of standard rabbit chow with 6.67% corn oil and 3.33% sodium chloride, and the low-salt groups were fed a diet that contained 0.23% sodium chloride. The lean groups were fed standard rabbit chow. The obese groups were given a high-fat diet ad libitum that consisted of standard rabbit chow with 6.67% corn oil and 3.33% sodium chloride. The rabbits were kept on their respective diets for 18 weeks. Using these dietary regimens, body weight, arterial blood pressure, hemodynamics, and blood sample analysis for the 4 groups of rabbits were as shown in Table 1. All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee at the University of Mississippi Medical Center and the American Physiological Society.

**Tissue Preparation**

On the day of the experiment, rabbits were anesthetized by inhalation of isoflurane. The thoracic aorta was rapidly removed, placed in oxygenated Krebs solution, and cleaned of connective tissue. The thoracic aorta was used in this study to maintain an acceptable level of accuracy and a small margin of error in the active stress and Ca\(^{2+}\) influx measurements. Because measurements of active stress and Ca\(^{2+}\) influx in vascular strips require normalization to the cross-sectional area and the weight of the individual strip, respectively, the margin of error in these measurements in a large multicellular preparation with a large cross-sectional area and appreciable weight, such as the thoracic aorta, are expected to be significantly less than that in a smaller resistance vessel with a small cross-sectional area and low weight. The aorta was cut into 3-mm-wide rings. Aortic rings were cut open into strips. The endothelium was removed by rubbing the vessel interior with forceps. Removal of the endothelium was routinely verified by the absence of acetylcholine (10\(^{-6}\) mol/L)-induced vasorelaxation in aortic strips precontracted with l-phenylephrine HCl (Phe; 3\(\times 10^{-7}\) mol/L).

**Isometric Tension**

One end of the aortic strip was attached to a glass hook using a thread loop, and the other end was connected to a Grass force transducer (FT03, Astro-Med). Aortic strips were stretched to 1.5 times initial unloaded length (L\(_u\)) and allowed to equilibrate for 1 hour in a water-jacketed, temperature-controlled tissue bath filled with 50 mL of Krebs solution continuously bubbled with 95% O\(_2\)/5% CO\(_2\) at 37°C. The changes in isometric tension were recorded on a Grass polygraph (model 7D, Astro-Med). Three different agonists were used in the present study. The \(\alpha\)-adrenergic agonist Phe was used to stimulate both Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) stores and Ca\(^{2+}\) entry from the extracellular space.\(^1\)\(^0\) Caffeine was used to activate the Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism in Ca\(^{2+}\)-free solution.\(^1\)\(^1\) Membrane depolarization by high KCl solution was used to activate the Ca\(^{2+}\) entry mechanism from the extracellular space.\(^1\)\(^0\) Preliminary concentration-response curves have shown that 10\(^{-5}\) mol/L Phe, 25 mmol/L caffeine, and 96 mmol/L KCl produce maximal responses in rabbit aortic strips. Therefore, the responses to these concentrations of Phe, caffeine, and KCl were used for comparison among the 4 groups of rabbits.

Two protocols were followed in the present study. In the first, a maximal control contraction to 10\(^{-5}\) mol/L Phe or 96 mmol/L KCl was elicited, and the tissue then was rinsed with Krebs solution 3 times for 10 minutes each. The bathing solution was changed to nominally 0% Ca\(^{2+}\) Krebs solution for 10 minutes and then was switched to Krebs solution containing different concentrations of extracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_e\); 0.1, 0.3, 0.6, 1.0, and 2.5 mmol/L) for 10 minutes. Either the tissues were stimulated with Phe (10\(^{-5}\) mol/L) or the bathing solution was changed to 96 mmol/L KCl solution containing different concentrations of [Ca\(^{2+}\)]\(_e\), and the contraction was allowed to reach a plateau level before the measurement was taken. In the second protocol, the tissues were incubated in normal Krebs solution (2.5 mmol/L Ca\(^{2+}\)) for 1 hour, transferred to Ca\(^{2+}\)-free (2 mmol/L EGTA) Krebs solution for 10 minutes, and then stimulated with Phe (10\(^{-5}\) mol/L) or caffeine (25 mmol/L) for 2 minutes or until the transient contraction returned to baseline.

**\(45\)Ca\(^{2+}\) Influx**

Aortic strips were incubated in normal Krebs solution for 1 hour, transferred to Krebs solution containing specific [Ca\(^{2+}\)]\(_e\) for 10 minutes, and then stimulated with Phe (10\(^{-5}\) mol/L) or 96 mmol/L}

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>3.7±0.1</td>
<td>5.5±0.24</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>85.0±1.0</td>
<td>96.0±2.0</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>177.0±6.0</td>
<td>220.0±7.0</td>
</tr>
<tr>
<td>Plasma sodium, meq/L</td>
<td>140.3±0.8</td>
<td>141.4±0.4</td>
</tr>
<tr>
<td>Plasma potassium, meq/L</td>
<td>4.15±0.06</td>
<td>3.87±0.06</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>141.9±2.7</td>
<td>162.4±2.9</td>
</tr>
<tr>
<td>Plasma insulin, (\mu)U/mL</td>
<td>15.5±1.0</td>
<td>32.5±3.4</td>
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Data represent mean±SEM of measurements in 4 to 17 rabbits per group.
KCl for 15 minutes. The tissues were transferred to the respective radioactive $^{45}$Ca$^{2+}$ (ICN Radiochemical)-labeled solution (specific activity, 2 $\mu$Ci/ml) for 90 seconds. The tissues were transferred to ice-cold Ca$^{2+}$-free (2 mmol/L EGTA) Krebs solution for 45 minutes to quench extracellular $^{45}$Ca$^{2+}$ label, as previously described. The tissues were weighed and placed in 2 mL of hypotonic (5 mmol/L) EDTA for 24 hours at 4°C to disrupt the cell membranes and release the intracellular content of $^{45}$Ca$^{2+}$. The next day, 4 mL of Ecolite scintillation cocktail was added, and the samples were counted in a scintillation counter (Beckman LS 6500, Beckman Instruments).

**Statistical Analysis**

The developed force was normalized for the cross-sectional area of each individual strip and expressed as active stress (N/m$^2$) by the following equation: Stress = Force/Cross-Sectional Area, where the intracellular content of $^{45}$Ca$^{2+}$. The next day, 4 mL of Ecolite scintillation cocktail was added, and the samples were counted in a scintillation counter (Beckman LS 6500, Beckman Instruments).

**Results**

Aortic strips from the 4 groups of rabbits showed increases in active stress to Phe ($10^{-5}$ mol/L) with increases in [Ca$^{2+}$], (Figure 1A). In lean rabbits on normal salt diet, Phe increased active stress from 1.2±0.3×10$^3$ N/m$^2$ (n=8) at 100 $\mu$mol/L [Ca$^{2+}$] to 14.1±1.6×10$^3$ N/m$^2$ (n=8) at 2.5 mmol/L [Ca$^{2+}$]. The Phe-induced stress in low-salt lean rabbits was significantly greater than that in normal-salt lean rabbits. In contrast, the Phe-induced stress in normal-salt obese rabbits was significantly less than that in normal-salt lean rabbits. On the other hand, the Phe-induced active stress in the low-salt obese rabbits was significantly enhanced to levels not significantly different from those in low-salt lean rabbits.

We investigated whether the differences in Phe-induced active stress among the 4 groups of rabbits reflected changes in Ca$^{2+}$ release from the intracellular stores. When Ca$^{2+}$-free (2 mmol/L EGTA) Krebs solution was used, Phe ($10^{-5}$ mol/L) and caffeine (25 mmol/L) caused a transient increase in active stress in aortic strips of normal-salt lean rabbits, which was not significantly different from that observed in aortic strips from low-salt lean, normal-salt obese, or low-salt obese rabbits (Table 2).

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We tested whether the observed differences in Phe-induced active stress reflected changes in the mechanisms of Ca$^{2+}$ entry. Membrane depolarization by high KCl is known to stimulate Ca$^{2+}$ entry from the extracellular space. All groups of rabbits showed increases in the 96-mmol/L KCl-induced active stress with increasing [Ca$^{2+}$], (Figure 1B). In normal-salt lean rabbits, KCl increased stress from 1.7±0.4×10$^3$ N/m$^2$ (n=8) at 100 $\mu$mol/L [Ca$^{2+}$] to 13.3±1.4×10$^3$ N/m$^2$ (n=8) at 2.5 mmol/L [Ca$^{2+}$]. The KCl-induced stress in low-salt lean rabbits was significantly greater than that in normal-salt lean rabbits. In contrast, the

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The normal Krebs solution contained (in mmol/L) NaCl 120, KCl 5.9, NaHCO$_3$ 25, NaH$_2$PO$_4$ 1.2, dextrose 11.5, MgCl$_2$ 1.2, and CaCl$_2$ 2.5, pH 7.4. For nominally 0% Ca$^{2+}$ Krebs solution, CaCl$_2$ was omitted. For Ca$^{2+}$-free Krebs solution, CaCl$_2$ was omitted and replaced with 2 mmol/L EGTA. The high-KCl depolarizing solution was prepared as Krebs solution but with equimolar substitution of KCl for NaCl. Stock solution of Phe (Sigma Chemical Co) was prepared as 10$^{-3}$ mol/L in distilled water. All other chemicals were of reagent grade or better.

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Aortic strips from the 4 groups of rabbits showed increases in active stress to Phe ($10^{-5}$ mol/L) with increases in [Ca$^{2+}$], (Figure 1A). In lean rabbits on normal salt diet, Phe increased active stress from 1.2±0.3×10$^3$ N/m$^2$ (n=8) at 100 $\mu$mol/L [Ca$^{2+}$] to 14.1±1.6×10$^3$ N/m$^2$ (n=8) at 2.5 mmol/L [Ca$^{2+}$]. The Phe-induced stress in low-salt lean rabbits was significantly greater than that in normal-salt lean rabbits. In contrast, the Phe-induced stress in normal-salt obese rabbits was significantly less than that in normal-salt lean rabbits. On the other hand, the Phe-induced active stress in the low-salt obese rabbits was significantly enhanced to levels not significantly different from those in low-salt lean rabbits.

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KCl-induced stress in normal-salt obese rabbits was significantly less than that in normal-salt lean rabbits. On the other hand, the KCl-induced stress in the low-salt obese rabbits was significantly enhanced to levels not significantly different from those in low-salt lean rabbits.

To investigate further whether the observed changes in active stress reflect changes in Ca\(^{2+}\) entry pathways, we measured the Phe-induced \(^{45}\text{Ca}^{2+}\) influx. All groups of rabbits showed increases in Phe-induced \(^{45}\text{Ca}^{2+}\) influx with increases in \([\text{Ca}^{2+}]_e\) (Figure 1C). In normal-salt lean rabbits, Phe (10\(^{-5}\) mol/L) increased \(^{45}\text{Ca}^{2+}\) influx from 4.78\pm0.85 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} (n=10) at 100 \mu\text{mol}/L \([\text{Ca}^{2+}]_e\), to 20.52\pm1.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} (n=10) at 2.5 mmol/L \([\text{Ca}^{2+}]_e\). The Phe-induced \(^{45}\text{Ca}^{2+}\) influx in low-salt lean rabbits was significantly greater than that in normal-salt lean rabbits. In contrast, the Phe-induced \(^{45}\text{Ca}^{2+}\) influx in normal-salt obese rabbits was significantly less than that in normal-salt lean rabbits. On the other hand, the Phe-induced \(^{45}\text{Ca}^{2+}\) influx in the low-salt obese rabbits was increased to levels indistinguishable from those in normal-salt lean rabbits.

To determine whether the observed changes in active stress reflect changes in \(^{45}\text{Ca}^{2+}\) entry through voltage-gated \(^{45}\text{Ca}^{2+}\) channels, we measured the KCl-induced \(^{45}\text{Ca}^{2+}\) influx. All groups of rabbits showed increases in KCl-induced \(^{45}\text{Ca}^{2+}\) influx with increases in \([\text{Ca}^{2+}]_e\) (Figure 1C). In normal-salt lean rabbits, KCl increased \(^{45}\text{Ca}^{2+}\) influx from 4.61\pm0.35 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} (n=10) at 100 \mu\text{mol}/L \([\text{Ca}^{2+}]_e\), to 26.4\pm1.68 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} (n=10) at 2.5 mmol/L \([\text{Ca}^{2+}]_e\). The KCl-induced \(^{45}\text{Ca}^{2+}\) influx in low salt lean rabbits was significantly greater than that in normal-salt lean rabbits. In contrast, the KCl-induced \(^{45}\text{Ca}^{2+}\) influx in normal-salt obese rabbits was significantly less than that in normal-salt lean rabbits. The KCl-induced \(^{45}\text{Ca}^{2+}\) influx in low-salt obese rabbits was enhanced to levels not significantly different from that in normal-salt lean rabbits.

To further investigate the possible \(^{45}\text{Ca}^{2+}\) entry pathways that might be involved in the Phe-induced changes in active stress, the Phe- and KCl-induced \(^{45}\text{Ca}^{2+}\) influx-active stress relationships were constructed and compared in each group of rabbits. If the low salt- or obesity-associated changes in vascular reactivity to Phe involve changes only in \(^{45}\text{Ca}^{2+}\) entry through voltage-gated \(^{45}\text{Ca}^{2+}\) channels, then the Phe-\(^{45}\text{Ca}^{2+}\) influx-stress relationship would be similar to that of KCl. In all groups of rabbits, the Phe-induced \(^{45}\text{Ca}^{2+}\) influx-stress relationship was located to the left of that induced by KCl (Figure 2). In other words, for the same level of \(^{45}\text{Ca}^{2+}\) influx, Phe caused greater active stress than KCl. In low-salt lean rabbits, the Phe-\(^{45}\text{Ca}^{2+}\) influx-stress relationship was significantly shifted to the left compared with that of KCl (Figure 2B). Also, a further shift to the left in the Phe-\(^{45}\text{Ca}^{2+}\) influx-stress relationship compared with that of KCl was observed in low-salt obese rabbits (Figure 2D).

To investigate whether other contractile mechanisms in addition to \(^{45}\text{Ca}^{2+}\) entry were involved in the observed changes in active stress, the Phe-induced \(^{45}\text{Ca}^{2+}\) influx-stress relationship was compared among the 4 groups of rabbits (Figure 3). If the observed changes in active stress associated with low-salt diet involve changes only to the \(^{45}\text{Ca}^{2+}\) entry mechanisms, then the \(^{45}\text{Ca}^{2+}\) influx-stress relationship in the low-salt rabbits would be different from, but would be an extension of, that in the normal salt rabbits. Likewise, if the observed changes in active stress associated with obesity involve changes only in the \(^{45}\text{Ca}^{2+}\) entry mechanisms, then the \(^{45}\text{Ca}^{2+}\) influx-stress relationship in obese rabbits would not be different from, but would be an extension of, that in lean rabbits. As shown in Figure 3A, the Phe-induced \(^{45}\text{Ca}^{2+}\) influx-stress relationship in low-salt lean rabbits was significantly greater than that in normal-salt lean rabbits. Although the Phe-\(^{45}\text{Ca}^{2+}\) influx-stress relationship in normal-salt obese rabbits was not significantly different from that in normal-salt lean rabbits, the Phe-\(^{45}\text{Ca}^{2+}\) influx-stress relationship in low-salt obese rabbits was significantly less than that in normal-salt lean rabbits.
The main findings of the present study are as follows: (1) low-sodium diet is associated with enhanced vascular reactivity in both lean and obese rabbits; (2) obesity is associated with reduced vascular reactivity in rabbits on normal-sodium diet; (3) low-salt- and obesity-induced changes in vascular reactivity are associated with changes in 
\[Ca^{2+}\] entry from the extracellular space but not 
\[Ca^{2+}\] release from the intracellular stores; and (4) the Phe-
\[Ca^{2+}\] influx–stress relationship is significantly enhanced by low-salt diet, particularly in obese rabbits.

The present study showed that the contractile response to Phe at increasing [\(Ca^{2+}\)]o was greater with low-salt than with normal-salt diet. Although the increased vascular reactivity to Phe with low-salt diet can, in part, be explained by an increase in the sensitivity to Phe at the \(\alpha\)-adrenergic receptor level, the enhanced vascular reactivity could also be due to stimulation of signaling mechanisms downstream from \(\alpha\)-adrenergic receptor activation. It is generally accepted that activation of \(\alpha\)-adrenergic receptors by agonists such as Phe causes activation of phospholipase C and increases the hydrolysis of phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. IP3 stimulates 
\[Ca^{2+}\] release from intracellular stores and diacylglycerol stimulates protein kinase C. In addition, \(\alpha\)-adrenergic agonists enhance 
\[Ca^{2+}\] entry through the plasma membrane 
\[Ca^{2+}\] channels.

We found that the transient Phe- and caffeine-induced contractions in 
\[Ca^{2+}\]-free solution, which are often used as a measure of IP3-induced 
\[Ca^{2+}\] release and 
\[Ca^{2+}\]entry from the intracellular 
\[Ca^{2+}\] stores, respectively, were not significantly different among the 4 groups of rabbits, which suggests that the enhanced vascular reactivity observed with low-salt diet is not due to changes in 
\[Ca^{2+}\] release from or 
\[Ca^{2+}\] uptake to the intracellular stores. On the other hand, these results showed that the Phe-induced 
\[Ca^{2+}\] influx was enhanced in rabbits on low-salt diet, which suggests enhancement of 
\[Ca^{2+}\] entry from the extracellular space. To investigate the possible 
\[Ca^{2+}\] entry pathways involved, we compared the Phe response with that induced by high KCl. High KCl is known to cause membrane depolarization and to stimulate 
\[Ca^{2+}\] entry through voltage-gated 
\[Ca^{2+}\] channels. We found that KCl-induced stress and 
\[Ca^{2+}\] influx were enhanced with low-salt diet, which provided evidence that 
\[Ca^{2+}\] entry from the extracellular space through voltage-gated 
\[Ca^{2+}\] channels may be enhanced. The observed increase in KCl-induced 
\[Ca^{2+}\] influx suggests that the 
\[Ca^{2+}\] permeability of voltage-gated 
\[Ca^{2+}\] channels is increased with a low-salt diet. However, other types of 
\[Ca^{2+}\] channels, such as the receptor-operated 
\[Ca^{2+}\] channels, may also be involved in the observed increase in the vascular reactivity to Phe with low-salt diet. The cause of the increased permeability of 
\[Ca^{2+}\] channels with low-salt diet is not clear at the present time but may be related to the possibility that low-salt diet is associated with increased renin release and increased circulating angiotensin II. On the other hand, an effect of low-salt diet on the Na+-
\[Ca^{2+}\] exchanger of different cells, including vascular smooth muscle cells, cannot be excluded under these conditions.

We found that obesity, with normal-salt diet, was associated with a decrease in vascular reactivity to Phe in rabbit aortic strips. Our results contrast with those of some studies, which have shown that the pressor response to angiotensin II in vivo as well as the vascular responsiveness to angiotensin II, Phe and serotonin are increased in obese Zucker rats. The cause of the difference between the results is not clear but may be related to the method of producing obesity in different animal species. The rabbit model that we used is produced by feeding the rabbits a high-fat diet, which often causes an increase in sympathetic activity. In contrast, the obese Zucker rat is characterized by a genetic abnormality of the leptin receptor in the hypothalamus and may not have marked increases in sympathetic activity. The decreased vascular reactivity to Phe in the obese rabbits in our study is consistent with previous reports of decreased vascular resistance and
increased regional blood flow in other animals made obese by feeding a high-fat diet.\textsuperscript{6,21}

The observed obesity-associated decrease in vascular reactivity can be explained by one of several mechanisms, such as decreased sensitivity to Phe at the \( \alpha \)-adrenergic receptor level, decreased vascular elasticity, or decreased \( \text{Ca}^{2+} \) mobilization into vascular smooth muscle. We did not find significant differences in the Phe- or caffeine-induced contractions, which suggests that \( \text{Ca}^{2+} \) release from and/or \( \text{Ca}^{2+} \) uptake to the intracellular stores is not different. On the other hand, we found significant reductions in Phe- and depolarization-induced \( \text{Ca}^{2+} \) influx, which suggests that the \( \text{Ca}^{2+} \) permeability of receptor-operated and voltage-gated \( \text{Ca}^{2+} \) channels may be decreased in obese animals. Because the obese rabbits were hyperinsulinemic, one possibility is that the decreased vascular reactivity and \( \text{Ca}^{2+} \) influx may be secondary to the effects of elevated insulin levels.

The present results also showed that in all groups of rabbits the Phe-induced \( \text{Ca}^{2+} \) influx-stress relationship is shifted to the left compared with that of KCl. If we assume that the depolarization-induced contraction is mainly due to stimulation of \( \text{Ca}^{2+} \) entry from the extracellular space, then the enhanced Phe response could be due to activation of contractile mechanisms in addition to \( \text{Ca}^{2+} \) entry. These possible mechanisms may include the following: (1) Phe may inhibit \( \text{Ca}^{2+} \) extrusion mechanisms such as the plasmalemmal \( \text{Ca}^{2+} \) pump and Na\textsuperscript{+}/\text{Ca}\textsuperscript{2+} exchanger, (2) Phe may disrupt superficially located \( \text{Ca}^{2+} \) buffering systems and thus allow more \( \text{Ca}^{2+} \) to be available for the myofilaments to cause contraction,\textsuperscript{22} and (3) Phe may increase the myofilament force sensitivity to \( \text{Ca}^{2+} \) or perhaps stimulate a completely \( \text{Ca}^{2+} \)-independent pathway. For example, Phe may activate protein kinase C through increased formation of diacylglycerol.\textsuperscript{15,23}

Because the Phe-induced \( \text{Ca}^{2+} \) influx-stress relationship was significantly greater than that of KCl in rabbits on low-salt diet, we can only suggest that one or more of these additional contractile mechanisms may be stimulated with low-salt diet.

To further investigate the possible contribution of mechanisms in addition to \( \text{Ca}^{2+} \) entry to the low-salt-associated changes in vascular reactivity, we compared the relationship between \( \text{Ca}^{2+} \) entry and active stress in low-salt versus normal-salt rabbits. If the low-salt-associated changes in active stress were merely due to changes in \( \text{Ca}^{2+} \) entry through plasma membrane \( \text{Ca}^{2+} \) channels, then one would not expect the \( \text{Ca}^{2+} \) entry-active stress relationship in low-salt rabbits to be different from that in normal-salt rabbits. The present study showed that the \( \text{Ca}^{2+} \) entry–stress relationship, particularly during stimulation with Phe, was greater in rabbits given low-salt compared with those given a normal-salt diet. These data further support the contention that contractile mechanisms in addition to stimulation of \( \text{Ca}^{2+} \) entry through plasma membrane \( \text{Ca}^{2+} \) channels are enhanced with low-salt diet.

In contrast to the enhanced \( \text{Ca}^{2+} \) entry observed in low-salt rabbits, we observed a reduction in \( \text{Ca}^{2+} \) entry in obese rabbits on normal-salt diet. If low salt and obesity were modulating the same \( \text{Ca}^{2+} \) entry mechanisms, one would predict that the simultaneous increase and decrease in \( \text{Ca}^{2+} \) entry would cancel each other out in obese rabbits on low-salt diet. Interestingly, our data show a rebound increase in the Phe-Ca\textsuperscript{2+} influx–stress relationship in obese rabbits on low-salt diet that is greater than that in lean rabbits on low-salt diets. These data can be explained by the possibility that Phe may further activate these additional contractile mechanisms in obese rabbits, possibly as a result of an increase in sympathetic activity with obesity.\textsuperscript{6} This is supported by reports that chronic blockade of \( \alpha \)- and \( \beta \)-adrenergic receptors markedly reduces blood pressure in hypertensive obese dogs\textsuperscript{21} and that renal denervation markedly attenuates the rise in blood pressure associated with induction of obesity.\textsuperscript{24}

Another possibility is that the increased levels of fatty acids associated with obesity\textsuperscript{6} may change the activity of lipid-sensitive enzymes such as protein kinase C.\textsuperscript{15}

Because the present study was performed on strips of thoracic aorta, we cannot make a definite conclusion as to whether the observed low-salt- and obesity-associated changes in the aorta also occur in resistance vessels; this is an important area for future investigation.

In conclusion, low-sodium diet is associated with enhanced vascular reactivity in both lean and obese rabbits. Obesity is associated with reduced vascular reactivity in rabbits on normal sodium diet. The low salt– and obesity-associated changes in vascular reactivity do not appear to involve changes in the \( \text{Ca}^{2+} \) release mechanisms but may involve significant changes in \( \text{Ca}^{2+} \) entry from the extracellular space. The enhanced \( \text{Ca}^{2+} \) influx–stress relationships with low-salt diet, particularly in obese rabbits, suggest activation of contractile mechanisms in addition to stimulation of \( \text{Ca}^{2+} \) entry. Further studies are needed to investigate these additional vascular contractile mechanisms, particularly during low-salt, high-fat diet regimens.

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Enhanced Vascular Reactivity and Ca\textsuperscript{2+} Entry With Low-Salt Diet: Effect of Obesity
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