Effect of Modest Salt Reduction on Skin Capillary Rarefaction in White, Black, and Asian Individuals With Mild Hypertension

Feng J. He, Maciej Marciniak, Nirmala D. Markandu, Tarek F. Antonios, Graham A. MacGregor

**Abstract**—Microvascular rarefaction occurs in hypertension. We carried out a 12-week randomized double-blind crossover trial to determine the effect of a modest reduction in salt intake on capillary rarefaction in 71 whites, 69 blacks, and 29 Asians with untreated mildly raised blood pressure. Both basal and maximal (during venous congestion) skin capillary density were measured by capillaroscopy at the dorsum and the side of the fingers. In addition, we used orthogonal polarization spectral imaging to measure skin capillary density at the dorsum of the fingers and the hand web. With a reduction in salt intake from 9.7 to 6.5 g/day, there was an increase in capillary density (capillaries per millimeter squared) from 101±21 to 106±23 (basal) and 108±22 to 115±22 (maximal) at the dorsum, and 101±25 to 107±26 (basal) and 110±26 to 116±26 (maximal) at the side of the fingers ($P<0.001$ for all). Orthogonal polarization spectral imaging also showed a significant increase in capillary density both at the dorsum of the fingers and the web. Subgroup analysis showed that most of the changes were significant in all of the ethnic groups. Furthermore, there was a significant relationship between the change in 24-hour urinary sodium and the change in capillary density at the side of the fingers. These results demonstrate that a modest reduction in salt intake, as currently recommended, improves both functional and structural capillary rarefactions that occur in hypertension, and a larger reduction in salt intake would have a greater effect. The increase in capillary density may possibly carry additional beneficial effects on target organs. *(Hypertension. 2010;56:253-259.)*

**Key Words:** sodium ■ dietary ■ microcirculation ■ capillary rarefaction ■ hypertension ■ randomized trial

**Microvascular rarefaction**, that is, a reduction in the number of arterioles and capillaries, is found in many animal models of hypertension and in human hypertension.1,2 We have shown previously that much of the reduction in capillary density in essential hypertension was because of the structural (anatomic) absence of capillaries,2 although functional capillary rarefaction (because of nonperfusion) also existed.3 Our previous studies also suggested that capillary rarefaction was likely to be a primary vascular abnormality.3,4 Microvascular rarefaction increases peripheral vascular resistance, thereby increasing blood pressure (BP) and aggravating BP-related target organ damage.1 At the same time, a reduction in the microvascular network may decrease tissue perfusion5 and cause gradual impairments of tissues and organs. Two studies have implied that long-term effective antihypertensive treatments may reverse microvascular rarefaction in nondiabetic individuals with raised BP.6–8 Studies in rats demonstrated that a high-salt intake caused a significant loss of microvessels,9,10 which occurred within a few days.10 However, no controlled trial in humans has studied whether a modest reduction in salt intake has an effect on microcirculation.

Direct intravital capillary videomicroscopy is a well-established method for studying skin capillaries.2,11 Because this technique depends on the presence of red blood cells inside capillaries, several procedures, for example, venous congestion, have been used to expose nonperfused capillaries. Therefore, the maximal capillary density during venous congestion reflects an anatomic number of capillaries. The limitation of capillaroscopy is that it is difficult to perform the measurement in individuals with thick and darkly pigmented skin. Orthogonal polarization spectral (OPS) imaging is a relatively new technique that allows direct visualization of the microcirculation in various human tissues and has the advantage of providing high contrast images as it uses the absorption of hemoglobin to visualize the microcirculation with a polarized light technique.12–14 Furthermore, the degree of magnification with OPS technique is approximately double that achieved by standard capillaroscopy.

The aim of our study was to determine the effect of a modest reduction in salt intake on skin capillary density in...
white, black, and Asian individuals with mildly raised BP. We used both capillaroscopy and OPS imaging to measure capillary density during a randomized, double-blind, placebo-controlled trial.

Methods
The methods of the study, including participants, study design, protocol, and measurements, were reported in detail elsewhere and summarized here. Individuals, aged 30 to 75 years, with sitting systolic BP 140 to 170 mm Hg or diastolic 90 to 105 mm Hg, and with no previous treatment for raised BP, were eligible. The study was approved by the Wandsworth Research Ethics Committee. Written consent was obtained from all of the participants.

After baseline assessments, which were made while on their usual diet, participants were advised to reduce their salt intake to ≈5 g/day (85 mmol/day). After 2 weeks on the reduced salt diet, participants entered the randomized double-blind crossover trial of slow sodium versus placebo but remained on the reduced-salt diet. They were allocated in random order to take 9 slow sodium tablets (10 mmol of sodium per tablet) or 9 placebo tablets daily for 6 weeks. They then crossed over to take the opposite tablets for 6 further weeks. All of the participants and research staff were unaware of the treatment allocation. BP, biochemistry, and capillary density measurements were performed at baseline and at the end of each 6-week period.

Capillary Density Measurement
After an overnight fast and 20-minute acclimatization in a temperature-controlled room (21°C to 24°C), measurements were performed in the morning with participants in the seated position and the left forearm and hand supported at heart level. Skin was cleaned, and a drop of liquid paraffin oil was used to reduce light reflection. Intravital capillaroscopy and orthogonal polarization spectral (OPS) imaging were performed in random order with a break of 15 minutes between the 2 measurements.

Intravital Capillaroscopy
Skin capillary density was measured according to a standardized technique as described previously. Microscopic images were obtained with a charge coupled device camera (Sony model XC-75CE) and were stored using a video recorder (JVC model HR-S6600). The skin of the dorsum and the side of the middle phalanx of the left hand were examined. Four microscopic fields (0.66 mm² per field) centered on an ink spot at each site were recorded continuously for 5 minutes to detect intermittently perfused capillaries. The number of capillaries per field was counted online and by running the recorded tapes using computer software (CapIScope, KK-Technology).

To maximize the number of visible capillaries, venous congestion was carried out. A miniature neonatal BP cuff was applied to the base of the left middle finger. The cuff was inflated and maintained at 60 mm Hg for 2 minutes. During the venous congestion, further images were recorded using 1 of the 4 microscopic fields chosen at random.

OPS Imaging
We used Cytoscan video microscopy to examine skin capillary density. Images from 4 random fields of the dorsum of the middle phalanx of the left hand were stored. In addition, 3 fields from the webbed skin between the index and middle finger, and the ring and middle finger, as well as the little and ring finger, were obtained. Each field (0.26 mm²) was continuously recorded for 3 minutes to identify intermittently perfused capillaries. Images were stored on a video recorder (JVC model HR-S6600). The number of capillaries per field was counted offline using computer software (KK-Technology).

Statistical Analysis
Paired Student t test was used to compare the difference between slow sodium and placebo for normally distributed variables. One nonnormally distributed variable (ie, plasma renin activity) was analyzed by Wilcoxon signed-ranks test. Potential carryover effects were examined using the methods described by Hills and Armitage. There was no significant carryover effect in any of the outcomes. Multiple linear regression analysis was carried out to test whether there was a significant relationship between 24-hour urinary sodium and capillary density with adjustment for confounding factors. A 2-tailed P<0.05 was regarded as statistically significant. All of the statistical analyses were performed using SPSS.

A total of 169 participants completed the study. There were 71 whites (56 men and 15 women), 69 blacks (34 men and 35 women), and 29 Asians (23 men and 6 women). Among the 29 Asians, 27 were of South Asian origin, 1 was Chinese, and 1 was of mixed ethnic origin (South Asian and white). Capillaroscopy was performed in 71 whites, 49 blacks, and 29 Asians at the dorsum of the fingers. This measurement could not be made in 20 blacks because of the darkly pigmented skin. In addition, 8 blacks had capillaroscopy performed at the side of the fingers. OPS imaging was carried out in 67 whites, 50 blacks, and 25 Asians at the dorsum of the fingers and in 68 whites, 21 blacks, and 10 Asians at the web.

Results
Results in All of the Participants
The mean age was 50±11 years, and baseline BP was 147±13/91±8 mm Hg. Other baseline characteristics and capillary density measurements are shown in Table 1. As expected, maximal capillary density (ie, during venous congestion) was higher than basal capillary density (ie, at resting).

From slow sodium to placebo, the mean salt intake was reduced from 9.7 to 6.5 g/day, that is, a reduction of 3.2 g/day as measured by 24-hour urinary sodium. With this reduction in salt intake, there was a significant fall in BP and a significant increase in plasma renin activity and aldosterone (Table 2).

Capillaroscopy data showed that, from slow sodium to placebo, there was a significant increase in both basal and maximal capillary density both at the dorsum and the side of the fingers (Table 2). At the dorsum of the fingers, basal capillary density was increased by 5%, and maximal capillary density was increased by 6%. At the side of the fingers, basal and maximal capillary density was increased by 6% and 5%, respectively. OPS imaging showed that, from slow sodium to placebo, there was an increase of 6% in capillary density at the dorsum of the fingers and 10% at the web (Table 2).

Results by Ethnic Group
Baseline data by ethnic group are shown in Table 1. From slow sodium to placebo, salt intake was reduced by 3.5 g/day in whites, 2.7 g/day in blacks, and 4.0 g/day in Asians. With these reductions in salt intake, there was a significant fall in BP in all 3 of the ethnic groups and a significant increase in plasma renin activity and aldosterone in whites only (Table 3).

Capillaroscopy data showed that, from slow sodium to placebo, there was a significant increase in most capillary density measurements in all 3 of the ethnic groups (Table 3). The increase in capillary density was between 3% and 5% for whites, 5% to 9% in blacks, and 7% to 9% in Asians. OPS
imaging showed that, from slow sodium to placebo, there was a significant increase in capillary density of 8% at the dorsum of the fingers and 9% at the web in whites. In blacks, there was a significant increase in capillary density of 10% at the web, whereas the increase in capillary density at the dorsum of the fingers (4%) was not statistically significant. In Asians, capillary density was increased by 8% (P<0.05) at the dorsum of the fingers and 16% at the web, the latter was not significant, but the number of individuals with this measurement was small (n=10).

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Participants</th>
<th>Whites</th>
<th>Blacks</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50 (11)</td>
<td>52 (12)</td>
<td>50 (9)</td>
<td>47 (10)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29 (5)</td>
<td>28 (5)</td>
<td>31 (5)</td>
<td>27 (5)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>147 (13)</td>
<td>146 (12)</td>
<td>149 (13)</td>
<td>142 (13)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>91 (8)</td>
<td>90 (8)</td>
<td>90 (8)</td>
<td>92 (10)</td>
</tr>
<tr>
<td>Urinary sodium, mmol/24 h</td>
<td>131 (50)</td>
<td>127 (51)</td>
<td>132 (46)</td>
<td>138 (57)</td>
</tr>
<tr>
<td>Plasma renin activity, ng/mL per h*</td>
<td>0.3 (0.1 to 0.6)</td>
<td>0.5 (0.3 to 0.9)</td>
<td>0.1 (0.1 to 0.2)</td>
<td>0.3 (0.1 to 0.5)</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>387 (175)</td>
<td>456 (179)</td>
<td>315 (145)</td>
<td>392 (167)</td>
</tr>
<tr>
<td>Hemoglobin level, mg/dL</td>
<td>14.6 (1.4)</td>
<td>15.0 (1.1)</td>
<td>14.0 (1.4)</td>
<td>15.1 (1.3)</td>
</tr>
</tbody>
</table>

Capillary density by capillaroscopy, capillaries per millimeter squared

<table>
<thead>
<tr>
<th>Dorsum of the fingers</th>
<th>All Participants</th>
<th>Whites</th>
<th>Blacks</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>At resting</td>
<td>102 (22)</td>
<td>106 (21)</td>
<td>100 (25)</td>
<td>94 (16)</td>
</tr>
<tr>
<td>With venous congestion</td>
<td>110 (24)</td>
<td>113 (24)</td>
<td>110 (26)</td>
<td>103 (17)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Side of the fingers</th>
<th>All Participants</th>
<th>Whites</th>
<th>Blacks</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>At resting</td>
<td>104 (24)</td>
<td>113 (23)</td>
<td>96 (23)</td>
<td>100 (21)</td>
</tr>
<tr>
<td>With venous congestion</td>
<td>112 (25)</td>
<td>119 (25)</td>
<td>106 (25)</td>
<td>108 (23)</td>
</tr>
</tbody>
</table>

Capillary density by OPS imaging, capillaries per millimeter squared

<table>
<thead>
<tr>
<th>Dorsum of the fingers</th>
<th>All Participants</th>
<th>Whites</th>
<th>Blacks</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>At resting</td>
<td>106 (23)</td>
<td>106 (22)</td>
<td>109 (24)</td>
<td>96 (18)</td>
</tr>
<tr>
<td>With venous congestion</td>
<td>88 (20)</td>
<td>88 (19)</td>
<td>92 (23)</td>
<td>83 (21)</td>
</tr>
</tbody>
</table>

All values are expressed as mean (SD) unless otherwise marked.
*Values are median (interquartile range).

Table 2. Changes in Variables From Slow Sodium to Placebo in All of the Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Slow Sodium</th>
<th>Placebo</th>
<th>Difference (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>146 (13)</td>
<td>141 (12)</td>
<td>-4.8 (-6.4 to -3.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>91 (8)</td>
<td>88 (9)</td>
<td>-2.2 (-3.1 to -1.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary sodium, mmol/24 h</td>
<td>165 (58)</td>
<td>110 (49)</td>
<td>-55 (-64 to -46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma renin activity, ng/mL per h*</td>
<td>0.12 (0.10–0.51)</td>
<td>0.23 (0.10–0.68)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>365 (175)</td>
<td>412 (187)</td>
<td>48 (26 to 70)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Capillary density by capillaroscopy, capillaries per millimeter squared

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<thead>
<tr>
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<th>All Participants</th>
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<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>At resting</td>
<td>101 (21)</td>
<td>106 (23)</td>
<td>4.9 (2.8 to 7.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>With venous congestion</td>
<td>108 (22)</td>
<td>115 (22)</td>
<td>6.3 (3.9 to 8.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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<thead>
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<td>107 (26)</td>
<td>6.3 (3.9 to 8.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>With venous congestion</td>
<td>110 (26)</td>
<td>116 (26)</td>
<td>5.8 (3.1 to 8.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Capillary density by OPS imaging, capillaries per millimeter squared

<table>
<thead>
<tr>
<th>Dorsum of the fingers</th>
<th>All Participants</th>
<th>Whites</th>
<th>Blacks</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>At resting</td>
<td>105 (24)</td>
<td>112 (25)</td>
<td>6.8 (3.9 to 9.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Web of the hand</td>
<td>83 (18)</td>
<td>92 (18)</td>
<td>8.4 (5.5 to 11.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are expressed as mean (SD) unless otherwise marked.
*Values are median (interquartile range).
Relationship Between Salt Intake and Capillary Density in All of the Participants

At baseline, that is, on participants’ usual diet, there was a significant inverse association between 24-hour urinary sodium excretion and capillary density at the side of the fingers (basal: \( r = -0.34, P < 0.001 \); maximal: \( r = -0.37, P < 0.001 \)), indicating the higher the salt intake, the lower the capillary density. These relationships were still significant after adjusting for age, sex, ethnic group, body mass index, and baseline mean arterial pressure. There was no significant association between 24-hour urinary sodium and any of the capillary density measurements.

During the randomized crossover phase, the change in 24-hour urinary sodium was significantly associated with the change in capillary density at the side of the fingers (basal: \( r = -0.38, P < 0.001 \), Figure 1; maximal: \( r = -0.37, P < 0.001 \), Figure 2), indicating the greater the reduction in salt intake, the greater the increase in capillary density. These relationships were still significant after adjusting for age, sex, ethnic group, body mass index, and the change in mean arterial pressure that occurred with salt reduction. There was also a significant association between the change in 24-hour urinary sodium and the change in capillary density at the dorsum of the fingers (basal: \( r = -0.19; P < 0.05 \)). This association became nonsignificant after adjusting for confounding factors (\( P = 0.084 \)). The relationship between the change in 24-hour urinary sodium and the change in basal capillary density at the dorsum of the fingers was not significant (\( r = -0.15; P = 0.070 \)).

Relationship Between BP and Capillary Density in All of the Participants

At baseline, there was no significant correlation between mean arterial pressure and any of the capillary density measurements. During the randomized crossover phase, the change in mean arterial pressure was significantly associated with the change in basal capillary density at the dorsum of the fingers (\( r = -0.19; P < 0.05 \)) and the change in maximal capillary density at the side of the fingers (\( r = -0.25; P < 0.01 \)). However, these relationships were not significant in multiple regression analyses where age, sex, ethnic group, body mass index, and the change in 24-hour urinary sodium were entered as independent variables. There was no significant correlation between the change in mean arterial pressure and the change in maximal capillary density at the dorsum of the fingers (\( r = -0.16; P = 0.062 \)) or the change in basal capillary density at the side of the fingers (\( r = -0.12; P = 0.126 \)).

Discussion

Our study, for the first time, demonstrates that a modest reduction in salt intake increases skin capillary density in white, black, and Asian individuals with mildly raised BP. The results are robust in that they are consistent for different areas of the skin and are consistent with 2 different measure-
ment methods. The finding of a significant relationship between the reduction in salt intake and the increase in capillary density suggests that a larger reduction in salt intake would have a greater effect.

Previous studies showed that both functional and structural skin capillary rarefactions occurred in individuals with raised BP.2,17 Our findings of an increase in capillary density at resting (functional) and during venous congestion (structural) indicate that a reduction in salt intake could improve both. Microvascular rarefaction contributes to the increased systemic vascular resistance in individuals with raised BP.1 Although it is uncertain to what extent skin microcirculation is involved in the regulation of systemic vascular resistance, it has been shown that microvascular rarefaction in hypertension occurs not only in the skin but also in other vascular beds, for example, skeletal muscle.10,18–21 In our study, the average salt intake was 9.7 g/day during the “normal” salt (slow sodium) period. This is very similar to the current salt intake in many countries around the world.22 During the reduced salt (placebo) period, the mean salt intake was 6.5 g/day, which is close to the United Kingdom recommended level of 6 g/day.23 Our results clearly suggest that this modest reduction in salt intake could improve microcirculation and reduce peripheral vascular resistance, thereby lowering BP and preventing vascular target organ damage.

Currently there is no direct evidence relating microvascular rarefaction to outcomes. However, a study by Paiardi et al24 has shown a correlation between microvascular rarefaction and media:lumen ratio of subcutaneous small resistance arteries, which has been demonstrated to be an independent predictor of cardiovascular events in hypertensive individuals.25,26 It is, therefore, possible that microvascular rarefaction may have deleterious effects on outcomes. It is speculated that, if microvascular rarefaction occurs in organs that hypertension is most likely to involve, that is, the brain, heart and kidney, it is possible that the reduction in microvascular network may cause further damage, particularly in reducing capillary reserve in these organs. This may contribute to a slow deterioration in the function of the brain, heart, and kidney, or, when some acute events occur, it is likely that greater structural damage may result if there is already a loss of microvascular network.

In our study, the increase in capillary density and the fall in BP occurred simultaneously. It is, therefore, difficult to know whether the increase in capillary density was the consequence or the cause of the BP fall or whether the 2 changes were independent of each other. However, experiments in rats have demonstrated that salt intake has a direct effect on microvascular circulation, independent of BP. For example, a study in Sprague-Dawley rats showed that, when salt intake was increased from 1 to 15 mmol/day for 3 days, there was no significant change in BP but there was a 24% reduction in microvascular density.10 Other studies with a longer duration have shown similar findings.9,19,27 It is possible that salt, rather than working through BP, may have a direct effect on microvascular network and could be one of the underlying causes for the microvascular rarefaction that occurs in hypertension.

The mechanisms whereby salt affects microcirculation are unclear. Previous studies have shown that a reduction in salt intake improves endothelial function.28,29 There is also evidence that endothelial dysfunction is related to microvascular rarefaction.1 Therefore, the improvement of endothelial function could be one of the mechanisms for the increase in capillary density with a reduction in salt intake. In addition,
an increase in plasma renin activity and thereby angiotensin II\(^{20}\) with a reduction in salt intake may play a role. It has been shown that angiotensin II, through its effect on angiotensin type 2 receptor, and bradykinin level, via bradykinin type 2 receptor, may affect the microvascular structure and function.\(^{1,30}\) In our study, there was a significant increase in plasma renin activity in whites only, whereas the increase in capillary density occurred in all 3 of the ethnic groups. It is not known how far the responses of the renin-angiotensin system contributed to the changes in capillary density. Further studies are needed to investigate the mechanisms.

The techniques including both capillaroscopy and OPS imaging used in our study were validated for measuring skin capillary density.\(^{2,11,14}\) Although capillaroscopy is of limited use in people with thick and dark skin, this technique issue will not affect the changes in capillary density during the randomized crossover period, because the same measurement methods were used throughout the whole study, and participants served as their own control in the crossover trial. The question is whether there is a difference between ethnic groups in capillary density and microvascular response to salt reduction; our study was not designed to address these questions. Other studies have suggested that blacks and South Asians had a poorer microvascular structure and function compared with whites, which could not be fully explained by conventional risk factors.\(^{31,32}\) However, a recent study using laser Doppler fluximetry in response to heating and ischemia showed that people of Indian Asian descent had a similar microvascular function as whites.\(^{33}\)

**Perspectives**

Our study demonstrates that a modest reduction in salt intake, as currently recommended in the United Kingdom and the United States, not only lowers BP but also increases skin capillary density in white, black, and Asian individuals with mildly raised BP. The finding of a significant relationship between the change in salt intake and the change in capillary density suggests that a larger reduction in salt intake would have a greater effect. The increase in capillary density in our study could be partially because of the fall in BP that occurred with salt reduction. However, experimental studies in rats have demonstrated that salt intake has a direct effect on microvascular density independent of BP.\(^{10}\) The increase in capillary density with a reduction in salt intake could reduce peripheral vascular resistance, thereby lowering BP. It may possibly have additional benefits on reducing organ damage. These results provide further support for the current recommendations to reduce salt intake to <6 g/day in adults.

**Acknowledgments**

We thank all of the participants; all of the staff in the Blood Pressure Unit who helped with recruitment and biochemical and BP measurements; and Healthspan Group, Ltd, for supplies of slow sodium and matching placebo tablets.

**Sources of Funding**

The study was in part funded by the United Kingdom Food Standards Agency (N02034).

**Disclosures**

None.

**References**


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Hypertension. 2010;56:253-259; originally published online June 28, 2010;
doi: 10.1161/HYPERTENSIONAHA.110.155747
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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