Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers

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ABSTRACT

Background: Japanese longevity is the highest in the world. This is partly explained by low occurrence of cardiovascular diseases, which in turn is attributed to the Japanese traditional diet (JTD). Recent research demonstrates that nitric oxide (NO), a key regulator of vascular integrity, can be generated from nitrate (NO₃⁻), abundantly found in vegetables. It can reduce blood pressure (BP) via its serial reduction to nitrite (NO₂⁻) and to bioactive NO. Interestingly, JTD is extremely rich in nitrate and the daily consumption is higher than in any other known diet.

Objective and design: In a randomized, cross-over trial we examined the effect of a 10-day period of JTD on blood pressure in 25 healthy volunteers. Traditional Japanese vegetables were encouraged to be consumed and avoided during the control period. Daily nitrate intake was calculated.

Results: Nitrate naturally provided by the JTD was 18.8 mg/kg/bw/day, exceeding the Acceptable Daily Intake by five times (ADI, 3.7 mg/kg/bw). Plasma and salivary levels of nitrate and nitrite were higher at the end of the JTD period. Diastolic BP decreased on average 4.5 mm Hg during JTD compared to the control diet (P = 0.0066) while systolic BP was not affected. This effect was evident in normotensive subjects and similar to that seen in the recent studies.

Conclusions: An ordinary nitrate rich diet may positively affect blood pressure. Our findings further support the importance of the role of dietary nitrate on BP regulation suggesting one possible explanation of healthy aspects of traditional Japanese food.

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Background

At an age when the average European person is predicted to die – 77 years for men and 81 for women – inhabitants of Okinawa in Japan usually have many more years of good health. Not only do the Japanese live longer, they age successfully, are lean, energetic and have low occurrence of chronic illnesses like heart disease and cancer [1]. This has partly been attributed to Japanese traditional diet rich in vegetables and fish. A typical Japanese meal consists of a rice dish complimented with soybean products, fish, seafood, and a variety of vegetables. Among the vegetables eaten every day, there are a variety of green leafy vegetables, mushrooms and seaweed. Interestingly, the population who lives longest (Okinawans), has the highest consumption of kombu (seaweed) in Japan [2]. Furthermore, the vegetable diet pattern in Japanese is associated with a significantly lower blood pressure, and serum triacylglycerides [3]. Specific foods that could reduce cardiovascular diseases have recently been identified [4–6] but more research is obviously required to identify what particular components in fruit and vegetables are associated with this decrease.

NO is a key regulator in vascular integrity. Recently a fundamentally different pathway for NO generation in addition to the classical NO synthase-dependent pathway has been described. NO can be generated from inorganic nitrate and nitrite, abundantly found in green leafy vegetables [7,8]. In humans, after absorption in the upper gastrointestinal tract, approximately 25% of circulating nitrate is actively taken up by the salivary glands and is concentrated up to 20-fold in saliva. Once in the oral cavity, commensal bacteria on the dorsal surface of the tongue reduce nitrate to nitrite by the action of nitrate reductase enzymes [8–10]. Swallowed nitrite is then reduced to NO and other bioactive nitrogen oxides in the acidic environment of the stomach. Nitrite that survives the acid conversion can enter the systemic circulation and increase its storage pool in blood and tissues. Studies in humans show increased plasma nitrite concentrations after oral ingestion of nitrate and use of an antibacterial mouthwash after
consumption of dietary nitrate attenuates the rise in plasma nitrite, showing the importance of the oral bacteria in the nitrate conversion to nitrite [11].

However, beyond this “prokaryotic pathway” of nitrite generation in blood and tissue an “eukaryotic pathway” has also been recently described by Lundberg’s group: also mammalian cells are capable of nitrate reduction to nitrite via the involvement of a nitrate reducing enzyme, XOR [12]. Nitrite accumulation in blood and tissues represent a biological pool for NO generation since several different mammalian enzymes and metalloproteins possess nitrite reductase activity such as xanthine oxidoreductase (XOR), aldehyde oxidase (AO), heme proteins and mitochondrial respiratory chain enzymes [11–13].

The measurable biological effects of nitrate derived NO include rapid local vasodilatation and acute reduction in blood pressure [5,13]. It also enhances gastroprotection [14–16], plays a role in mitochondrial respiration [17], cardiac function [18] and exerts antiapoptotic effects [19].

Significant physiologic benefits may be associated with the dietary nitrate. The content of inorganic nitrate in certain vegetables and fruits can provide a physiological substrate for reduction to nitrite and NO that produces vasodilatation, decreases blood pressure and supports cardiovascular function [20–22].

The Dietary Approaches to Stop Hypertension (DASH) studies found that diets rich in vegetables can lower blood pressure to levels similar to those achieved with single hypotensive medications [23,24]. This protection has been attributed to the high content of antioxidants, yet large clinical trials have failed to provide evidence in support of this theory [25,26]. The strongest protection against coronary heart disease was associated with the consumption of green leafy vegetables (e.g., spinach, lettuce) [27]. These vegetables commonly have a high inorganic nitrate content [28]. Interestingly, the BP reduction described after ingestion of beetroot juice decreased BP only if saliva was continuously swallowed, demonstrating the critical involvement of an enteroisolary circulation of nitrate for its bioactivation [5]. Other foods rich in nitrate–nitrite are mushrooms and seaweed. Asian population, especially Japanese, consume a diverse range of mushrooms and seaweed on a daily basis. Overall, the traditional Japanese diet contains a great number of green, leafy vegetables, making it exceptionally rich in nitrate, and the daily consumption higher than in any other known diet. We therefore aimed to examine if the Japanese traditional food, reflected in ingestion of dietary nitrate, affects plasma nitrate/nitrite and arterial blood pressure.

### Experimental procedures

The 25 participants of the study were physically active, healthy Japanese volunteers (10 men and 15 women; mean age 36 ± 10 years, BMI < 18.5). They gave informed consent and the study was granted full ethics approval by the Local Research Ethics Committee at Kyorin University School of Medicine and was registered at clinicaltrials.gov, NCT 00928824. The study had a randomized cross-over design with two dietary intervention periods during which the subjects received either Japanese or control (non-Japanese) diet. The exclusion criteria were any serious ill-health and any known diet. We therefore aimed to examine if the Japanese traditional food, reflected in ingestion of dietary nitrate, affects plasma nitrate/nitrite and arterial blood pressure.

**Table 1** Example of nitrate levels (NO3⁻, mg/kg) in some typical Japanese foods included in daily diets, based on dietary recall [29].

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>NO3⁻, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta cai</td>
<td>5670 ± 1270</td>
</tr>
<tr>
<td>Chin gai</td>
<td>3150 ± 1700</td>
</tr>
<tr>
<td>Garland chrisantemum</td>
<td>4410 ± 1455</td>
</tr>
<tr>
<td>Osaka shirona</td>
<td>2500 ± 753</td>
</tr>
<tr>
<td>Spinach</td>
<td>3560 ± 552</td>
</tr>
<tr>
<td>Burdock</td>
<td>2350 ± 438</td>
</tr>
<tr>
<td>Sayaingen beans</td>
<td>945 ± 141</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>1040 ± 289</td>
</tr>
<tr>
<td>Winter mushrooms</td>
<td>983 ± 93</td>
</tr>
<tr>
<td>Honghimeji mushrooms</td>
<td>1836 ± 48</td>
</tr>
<tr>
<td>Shitake mushrooms</td>
<td>454 ± 38</td>
</tr>
<tr>
<td>Purple laver</td>
<td>2825 ± 2200</td>
</tr>
<tr>
<td>Laver</td>
<td>3950 ± 3940</td>
</tr>
<tr>
<td>Nozavana pickles</td>
<td>2170 ± 35</td>
</tr>
<tr>
<td>Water dropwort</td>
<td>504 ± 187</td>
</tr>
</tbody>
</table>

Healthy Japanese individuals participating in the study followed proposed dietary schemes without significantly loosing or gaining body weight (mean ±SD), 58.7 ± 9.3 at the start of the trial, 58.9 ± 9.1 kg after the non-Japanese and 58.7 ± 9.3 after the Japanese diet). The trial subjects did not express any inconvenience fol-
lowing the Japanese diet; on the contrary, it was associated with an old-fashioned Japanese diet consumed at participants’ parents/ grandparents homes. At the same time it was difficult to follow the control diet. Therefore, a dietary expertise was used to adjust the control diet and to ensure its nitrate levels to be within the ADI range. Individually consumed daily nitrate intake was approximated to a mean concentration of 18.8 mg/kg of body weight/day during the Japanese diet study phase. Nitrate, naturally derived from Japanese diet exceeded five times the Acceptable Daily Intake (ADI = 3.7 mg/kg/body weight).

After 10 days of Japanese diet, the circulating plasma nitrate levels were higher than after period of control diet (mean [±SD], 43.2 ± 17.4 and 153.9 ± 149 μM, respectively; P < 0.001), as were plasma nitrite levels (131.5 ± 75.34 and 203.5 ± 102.3 nM, respectively; P = 0.0063) (Fig. 1). Fasting salivary nitrate levels were (median [range], 569.6 (14.4–5778) μM after Japanese diet (P = 0.0008) and 199.7(0.1–703.7) μM after control; nitrite levels were 134.2 (1.2–1411) μM at the end of the Japanese diet, and 71.9 (0.4–453.2) μM at the end of the control phase P < 0.0018.

The mean diastolic blood pressure was 4.5 mm Hg lower after Japanese diet compared with non-Japanese diet, 71.3 ± 7.9 and 75.8 ± 7.8, P = 0.0066) (Fig. 2). There were no significant differences in systolic blood pressure (data not shown).

Discussion

Until recently, it has been commonly agreed that NO in vivo could only be synthesised by NOS with nitrite and nitrate as inert biological end-products of NO metabolism. However, it was demonstrated in 1994, that nitrite derived from dietary nitrate was a substrate for NOS-independent generation of NO in the acidic condition of the human stomach [8,9]. Despite the demonstration of a pharmacological role for nitrite in vascular and immune function, the potential health aspects of food sources of nitrates and nitrites have not received much attention [22].

The WHO reported in 2002 that the harmful effects of chronic hypertension stand for the ca 11% of all following diseases. Identifying dietary components that might protect against cardiovascular diseases will therefore be important for public health worldwide. Nitrate has been highlighted to be such a component [5,14]: administration of sodium nitrate (0.1 mmol/kg/d) to healthy volunteers over 3 days reduced diastolic BP by 3.7 mm Hg [13] and Webb and co-workers showed similar effects with a vegetable juice rich in nitrate [5]. In the present study, ordinary Japanese diet increased intravascular stores of nitrite probably.
due to bioconversion. As a result, the BP decreased, because the nitrate was further converted to a potent vasodilator, NO [32]. Blood pressure decrease in normotensive Japanese volunteers was similar to that seen in the Webb and Larsen studies [5,13,23] and suggests that NO provided in the form of dietary nitrate, found in the Japanese traditional diet, would likely have a cardioprotective effect.

It is argued that the BP lowering effect of Japanese foods could be attributed to antioxidants, vitamins, polyphenols and high K⁺ content of fruit and vegetables [33], although recent large scale clinical trials have failed to provide evidence in support of this hypothesis [25,26]. At the same time, nitrite reduction to NO is greatly enhanced by reducing compounds such as vitamin C and polyphenols, both of which are abundant in the Japanese foods and in the DASH diet.

Further, Webb and co-workers elegantly showed that the lowering effect of vegetable juice on BP was independent of K⁺ levels, since the rise in plasma K⁺ was unaffected by spitting, while nitrate effect on BP were abolished by these procedure [5]. Moreover, since dietary sodium nitrate supplementation in the present study has similar effects as shown by Larsen et al. [13], this convincingly suggests that it is nitrate and not antioxidant, polyphenols or potassium that is responsible for the BP effect.

In the present study the amount of nitrate naturally provided by the Japanese diet exceeded the ADI by four times and could therefore be questioned. Although seemingly high, these levels were easy to reach when the participants ate vegetables that corresponded to a typical traditional Japanese diet. Green leafy vegetables present in Japanese food (chingsensai, komatsuna and Garland chrisanthemum etc.) contain on average a similar amount of nitrate as European spinach, and Japanese are high consumers of a variety of mushrooms and seaweed, also rich in nitrate/nitrite (Table 1). The variety and amounts of nitrate rich vegetables eaten every day in the traditional Japanese diet is much greater than in a European diet: almost all the foods shown in Table 1 were included in the daily diet, which corresponded an ordinary Japanese diet. Altogether, these eating habits explain the high daily intake of nitrate. Nitrate intake from dietary sources in our study is similar with the Japanese traditional diet, would likely have a cardioprotective effect. By highlighting the daily nitrate and nitrite contents of vegetables our study strengthens the existing evidence to advise vegetable consumption for health benefits. Time might have come to re-evaluate the ADI recommendations regarding nitrate consumption.

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