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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 (NO_3^-), abundantly found in vegetables. It can reduce blood pressure (BP) via its serial reduction to nitrite (NO_2^-) 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.

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

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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 enterosalivary 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 illnesses, infectious diseases and use of systemic medication. Study subjects had an overnight fast on the morning of saliva and blood collection and blood pressure recording. There were two smoking participants and they were instructed not to change their smoking habits during the study period.

Common Japanese vegetables, identified as the daily source of nitrate [29,30], were encouraged to consume during the consequent 10 days. To avoid the concentration differences in nitrate/nitrite in locally produced foods, the participants were provided with fresh vegetables and staple foods from the same store twice a week during both intervention periods. During the control period, participants were instructed to avoid these vegetables. Instead, the study subjects received and followed the instructions how to replace traditional Japanese meals; for example, by having cornflakes, muesli, yogurt or sandwiches for breakfast. The control diet was designed to eliminate the risk of any major differences between diets in total protein, carbohydrates, saturated and unsaturated fat. The control diet was controlled for nitrate and nitrite sources and those were excluded from the consumption. The mean total intake of energy was approximately 1900 kcal per day. Each participant was asked to recall and fill in all food and drink items consumed daily during the study periods of 10 days, including detailed information about recipe ingredients. Daily nitrate/nitrite intake calculations were based on the dietary recall, body weight information and referred nitrate/nitrite concentrations of ingested foods [29]. Nitrate contents in various vegetables and Japanese foods (examples are shown in Table 1) were based on the calculations by Tsuji et al. (assessed by ion exchange HPLC–UV chromatography) [29]. Blood pressure was measured manually in a sitting position by the blinded physician according to a standard protocol. There was no wash-out period between the study periods. Measurements were taken before breakfast three times: at baseline, at the shift of diets in the middle and at the end of the study. Blood samples (3 mL) were collected and treated for plasma nitrate and nitrite measurement according to procedure previously described in detail [31]. Blood samples were centrifuged immediately at 2200*g* for 10 min at 4 °C and the plasma stored at −80 °C until measurement of nitrite and nitrate concentration. Saliva samples were collected at the same time in tubes containing EDTA (final concentration 2 mM) and stored at −80 °C for later nitrate and nitrite determination. Samples were analyzed for nitrite and nitrate concentrations by ion chromatography (ENO 20 Analyzer; Eicom, Kyoto, Japan). Sample concentrations reflect the mean value from triplicate analyses.

The data were analyzed using the Graph Pad Prism Software. Group differences were tested with Mann–Whitney (independent groups) and Wilcoxon's signed rank (paired measurements) tests. In all cases, $P < 0.05$ was considered statistically significant.

Results

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-

Table 1
Example of nitrate levels (NO_3^-, mg/kg) in some typical Japanese foods included in daily diets, based on dietary recall [29].

Vegetable	$NO3^-$, mg/kg
Ta cai	5670 ± 1270
Chin gin cai	3150 ± 1760
Garland chrisantemum	4410 ± 1455
Osaka shirona	2500 ± 753
Spinach	3560 ± 552
Burdock	2350 ± 438
Sayaingen beans	945 ± 141
Chinese cabbage	1040 ± 289
Winter mushrooms	983 ± 93
Honghimeji mushrooms	1836 ± 48
Shiitake mushrooms	454 ± 38
Purple laver	2825 ± 2200
Laver	3990 ± 3940
Nozavana pickles	2170 ± 35
Water dropwort	504 ± 187

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).

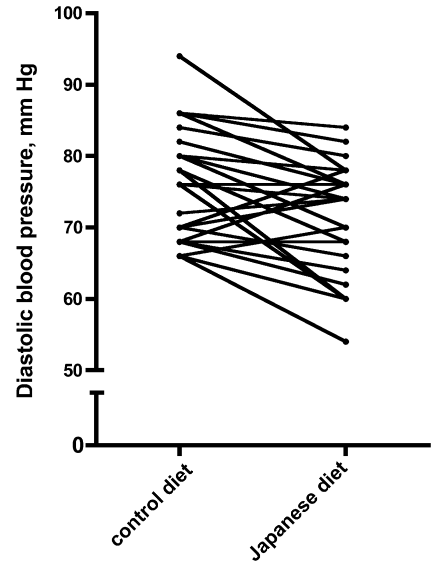

Fig. 2. Effects of 10-Day Japanese traditional foods or control diet on diastolic blood in 25 healthy volunteers. The mean diastolic pressure was by 4.5 mm Hg lower during Japanese traditional food intake ($P = 0.0066$).

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 an 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

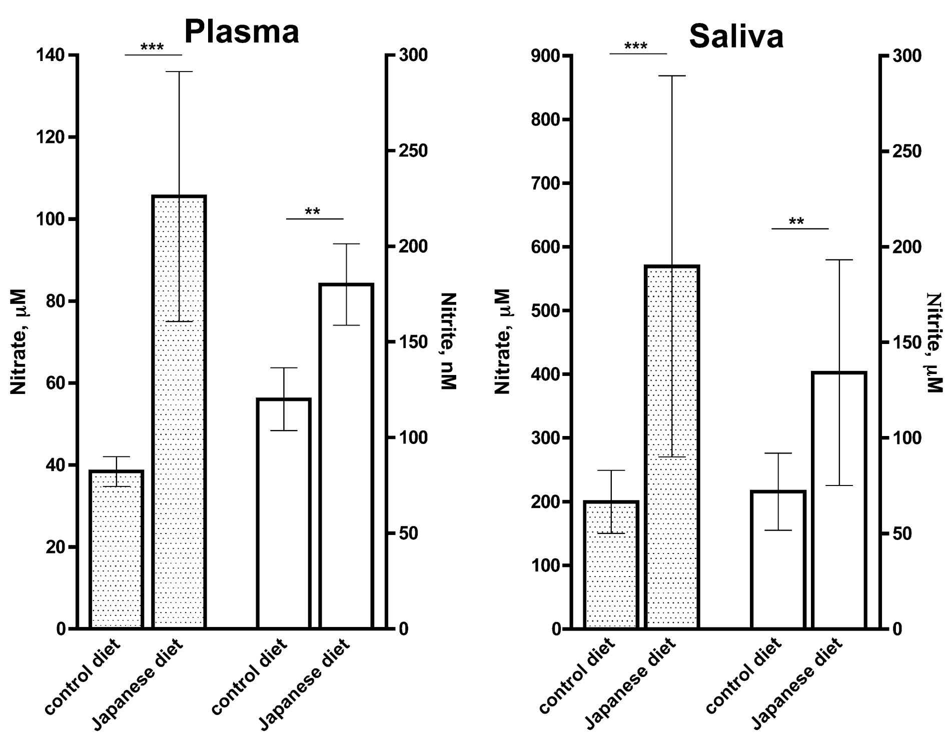

Fig. 1. Effects of 10-Day Japanese traditional foods or control food on salivary and plasma nitrate and nitrite in 25 healthy volunteers. After overnight-fasting (10 h), plasma nitrate and nitrite concentrations were higher during Japanese traditional diet ($P < 0.001$ and $P < 0.05$).

due to bioconversion. As a result, the BP decreased, because the nitrite 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 an 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 (chingensai, komatsuna and garland chrisantemum 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 recent report from Bryan's group, who has calculated that the DASH diet could result in the consumption of up to 1222 mg nitrate per day thereby exceeding by 550% the WHO's ADI for nitrate in adults [34]. The concentration of nitrate in a single vegetable species varies depending on the soil and growth as well as storage and transport conditions [35]. In our study, we handled the possible variation in concentrations by providing the participants with the foods from the same store.

Some nitrogenous compounds, such as nitrate, since long have been and still are considered potential human health hazards; especially when given to infants, nitrates in bacterially contaminated well water could be reduced to nitrite and cause the condition known as methemoglobinemia [20]. However, the exposure studies on children and adults have not confirmed that nitrate intake is associated with methemoglobinemia [36,37], and alternative explanations for methemoglobinemia in infants has been suggested such as gastroenteritis and associated iNOS-mediated production of nitric oxide induced by bacteria contaminated water [38]. The data supporting the toxicity of nitrates and nitrites for healthy adolescent and adult populations is questionable, as is the scientific basis for exposure regulations for nitrate and nitrite [20,21,39]. Another issue, especially in Japan, is that ingested nitrites may react with secondary amines or *N*-alkylamides to generate carcinogenic *N*-nitroso compounds (NOCs) [40] and the prevalence of gastric cancer in the Japanese population is very high. Although shown in animal models [41], the proof in humans has not been substantiated. Furthermore, the nitrites in foods may be "neutralized" if accompanied by vitamin C, an antioxidant that inhibits the nitrosation effect of nitrites on secondary amines [42]. Clearly, more research is needed to address whether nitrate and nitrite intake is associated with increased cancer risk.

Today we are facing a paradigm shift, and the recent description of the vasoprotective, blood pressure-lowering, and antiplatelet aggregation properties of nitrite suggests that a re-examination of these health effects would be beneficial [5,43,44]. Of the recent studies describes increased plasma nitrite and nitrate concentrations of natives in the high-altitude of Tibet as a natural physiology not associated with harmful physiological effects [45]. The DASH diet study led to the public dietary health recommendations in the United States [46].

We are aware of our study's limitations and the findings therefore should be generalized cautiously. First, the sample size was small. Second, the compliance was difficult to access, since the dietary recalls do not always guarantee accuracy due the risk of underreporting. Despite of this, the strength of the present study is that it is an ordinary diet and could therefore be recommended as a preventive strategy if further confirmed in longer studies also including subjects with cardiometabolic risk factors.

We conclude that Japanese traditional diet, rich in nitrate reduces diastolic blood pressure in healthy volunteers. Our results show effects of an easy to follow, diverse diet and suggest a possible explanation of healthy aspects of Japanese food. 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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