Vascular dilatory functions of ovo-lactovegetarians compared with omnivores

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Abstract

Vegetarians have lower blood pressure and lower cardiovascular mortality. Vegetarian diets may have lower cardiovascular risks through positive influence on endothelium-dependent relaxation and related functions. The objectives of this study were to assess the differences of vascular dilatory functions between middle-aged vegetarians and sex and age-matched omnivores before they develop any clinical manifestations of atherosclerosis. Twenty healthy vegetarians over the age of 50 and 20 healthy omnivores over the age of 50 were recruited for this study. Subjects with known risk factors for atherosclerosis such as hypertension, diabetes, obesity, hypercholesteremia, cigarette smoking, family history of vascular diseases, or taking any regular medication were excluded. Medical history, body weight, height, and duration of vegetarian diet were recorded. Baseline CBC, urinalysis and biochemical data such as fasting blood glucose, thyroid function, blood urea nitrogen, creatinine, serum electrolytes (sodium, potassium, chloride, calcium and magnesium), lipid profiles [total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol] were obtained after a 14 h fast. Blood pressures and heart rate were recorded in supine position. Vascular dilatory functions, both flow-mediated (endothelium-dependent) and nitroglycerin-induced (endothelium-independent), were evaluated using a non-invasive ultrasonographic method. The results show that there were no significant differences in the baseline characteristic between the vegetarians and the omnivores. There were also no significant differences in serum glucose, lipid profiles and thyroid function between these two groups. However, vasodilatation responses (both flow-mediated and nitroglycerin-induced) were significantly better in the vegetarian group and the degree of vasodilatation appeared to be correlated with years on vegetarian diets. Our findings suggest that vegetarian diets, by themselves, have a direct beneficial effect on vascular endothelial and smooth muscle function and may help to account for the lower incidence of atherosclerosis and cardiovascular mortality. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Vegetarian; Endothelium; Vascular function; Atherosclerosis

1. Introduction

The health benefits of vegetarian diets have been well documented [1]. As compared with omnivores, vegetarians have lower blood pressure [2] and lower cardiovascular mortality [3,4]. However, the exact mechanisms of health benefits of vegetarian diets on cardiovascular diseases remain unclear.

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The endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide (NO) [5–7], is a potent endogenous vasodilator that also inhibits platelet adhesion and aggregation, monocyte adherence and chemotaxis and hence has the potential to be antiatherogenic [8]. Palmer et al. reported that dietary arginine is a precursor of nitric oxide in vascular endothelium [9]. Oxidized low density lipoproteins inhibits the effect of EDRF and fed arginine in doses equivalent to those commonly obtained from certain vegetable foods (e.g. nuts) corrects the reduced concentrations of EDRF in hypercholesteremic animals [10].
Large-scale dietary study indeed suggests that frequent consumption of nuts may be protective for both fatal and non-fatal coronary event [11]. These findings suggest that vegetarian diets may have lower cardiovascular risks through positive influence on endothelium-dependent relaxation and related functions such as platelet aggregation and adhesion, proliferation of vascular smooth muscle cells, etc.

 Vasodilatory response to exogenous nitroglycerin (NTG) has also been found to be impaired in asymptomatic subjects with high cholesterol, cigarette smoking, diabetes mellitus (DM), increased age, male gender, larger vessel size, and reduced endothelium-dependent dilation [12,13]. The impairment of vasodilatory functions, both endothelium-dependent and independent, is an important early event in atherogenesis, preceding formation of plaques [12].

In Chinese society, vegetarianism originates from the Buddhist's teaching of 'no killing' and has been practiced for centuries. Most people eat dairy products and eggs in addition to plant food (lacto-ovo-vegetarians) [14]. Some members at The Buddhist Tzu Chi Compassion Relief Foundation followed the teaching and became lacto-ovo-vegetarians who eat no meat, poultry or fish. In Taiwan, vegetarians consumed less calories except for the males, more carbohydrate (63% of energy in men, 58% in women), less protein (12% of energy) and fat (25% for men, 30% for women) [15,16]. The mean polyunsaturated/saturated fatty acid (P/S) ratio in vegetarians is about two or three times that in omnivore diet [15,16].

Thus the aim of this present study was to examine the differences of vascular dilatory functions between this group of vegetarians and omnivores before they develop clinical manifestations of atherosclerosis by using a non-invasive ultrasonographic method, which have been demonstrated to be accurate and reproducible [17,18].

2. Subjects and methods

Twenty volunteers (10 male, 10 female) who have been on a vegetarian diet for at least 1 year and are 30 years or older (since vascular diseases are uncommon under 50 years of age) were recruited from the Buddhist Tzu Chi Compassion Relief Foundation. A 20-omnivore control group (10 male, 10 female) was also recruited from the same foundation. Subjects with smoking, alcoholism, family history of vascular diseases, or prior history of diseases that can affect cardiovascular systems such as hypertension (HTN), DM, hyperlipoproteinemia, and overt vascular diseases such as cerebrovascular diseases, angina pectoris, myocardial infarction, etc were excluded from this study. This was expected to be sufficient to demonstrated dietary effects on vascular dilatory functions. Appropriate committees of the hospital approved all the studies and each subject gave informed consent.

Table 1 summarizes the characteristics of both vegetarian and omnivore volunteers. The vegetarians are somewhat older (not statistically significant, P = 0.073), they had been on vegetarian diets for 2–21 years with average of 7.9 ± 5.1 years. There were no significant baseline differences in body mass index, systolic blood pressure, diastolic blood pressure, resting heart rate. BUN, serum creatinine, and serum electrolytes.

Blood biochemistry and thyroid function of vegetarian and omnivore volunteers are listed in Table 2. There were no significant differences in serum glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, T3, T4, and TSH between these two groups.

2.1. Study design

Medical history, body weight, height, and duration of lacto-vegetarian diet were recorded and baseline CBC, urinalysis and biochemical data such as fasting

<table>
<thead>
<tr>
<th>Table 1</th>
<th>omnivore (n = 20)</th>
<th>vegetarian (n = 20)</th>
<th>t-test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean ± S.D.*, n)</td>
<td>56.4 ± 4.0</td>
<td>58.6 ± 3.5</td>
<td>0.073</td>
</tr>
<tr>
<td>Body mass index (Kg/m²)</td>
<td>24.8 ± 1.1</td>
<td>23.1 ± 3.1</td>
<td>0.085</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>121 ± 8</td>
<td>123 ± 15</td>
<td>0.672</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>78 ± 8</td>
<td>77 ± 9</td>
<td>0.602</td>
</tr>
<tr>
<td>Heart rate (Beats/min)</td>
<td>66 ± 8</td>
<td>65 ± 8</td>
<td>0.612</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>11.2 ± 2.6</td>
<td>12.4 ± 2.4</td>
<td>0.159</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.210</td>
</tr>
<tr>
<td>Serum sodium (mmol/l)</td>
<td>142.4 ± 1.6</td>
<td>141.4 ± 1.3</td>
<td>0.067</td>
</tr>
<tr>
<td>Serum potassium (mmol/l)</td>
<td>4.3 ± 0.4</td>
<td>4.5 ± 0.8</td>
<td>0.205</td>
</tr>
<tr>
<td>Serum chloride (mmol/l)</td>
<td>105.7 ± 2.0</td>
<td>105.3 ± 1.7</td>
<td>0.413</td>
</tr>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>2.2 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>0.726</td>
</tr>
<tr>
<td>Serum magnesium (mmol/l)</td>
<td>2.2 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>0.823</td>
</tr>
</tbody>
</table>

* S.D., standard deviation.
Table 2
Blood biochemistry and thyroid function of vegetarian and omnivore volunteers*a,b

<table>
<thead>
<tr>
<th></th>
<th>Omnivore (n = 20)</th>
<th>Vegetarian (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>98 ± 14</td>
<td>87 ± 8</td>
<td>0.655</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>166 ± 36</td>
<td>162 ± 32</td>
<td>0.694</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>120 ± 30</td>
<td>116 ± 29</td>
<td>0.617</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>46 ± 17</td>
<td>48 ± 8</td>
<td>0.664</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>100 ± 51</td>
<td>94 ± 33</td>
<td>0.655</td>
</tr>
<tr>
<td>T₃ (ng/dl)</td>
<td>118 ± 28</td>
<td>116 ± 23</td>
<td>0.816</td>
</tr>
<tr>
<td>T₄ (μg/dl)</td>
<td>8.3 ± 1.4</td>
<td>8.3 ± 1.7</td>
<td>0.864</td>
</tr>
<tr>
<td>TSH (μIU/ml)</td>
<td>1.5 ± 0.9</td>
<td>1.4 ± 0.6</td>
<td>0.693</td>
</tr>
</tbody>
</table>

*a All values are expressed as mean ± standard deviation
b LDL, low density lipoprotein; HDL, high density lipoprotein; T₃, triiodo-L-thyronine; T₄, L-thyroxine; TSH, thyroid-stimulating hormone.

blood glucose, thyroid function [L-thyroxine (T₄), triiodo-L-thyronine (T₃), Thyroid-stimulating hormone (TSH)], blood urea nitrogen (BUN), creatinine, serum electrolytes (sodium, potassium, chloride, calcium and magnesium), lipid profiles [total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol] were obtained after a 14 h fast. Blood pressures, heart rate were recorded in supine position. The mean of three readings in every 10 min measurement was used as the data of blood pressure and heart rate for the subject.

The vasodilatory functions, both flow-mediated and NTG-induced were evaluated according to the method described by Sorensen et al. and Celermajer et al. as described below [17,18]. The results were analyzed and statistical significance calculated. Student t-tests were used to compare means between the two groups. Multivariate analysis, Linear regression and Pearson correlation coefficient calculation were performed using Microsoft Excel and SAS software (SAS Institute, SAS Campus Drive, Cary, NC). A P value of < 0.05 was considered statistically significant.

2.2. Test for flow-mediated and NTG-induced vasodilation [17,18]

The subject rested in the supine position for 10 min before the first scan and remained supine until the final recording was acquired. An Acuson Ultrasound imaging machine with a 7.0 MHz linear array transducer (Acuson, Mountain View, CA) was used to obtain ultrasound images of the right brachial artery. The center of the vessel was identified when the clearest images of the anterior and posterior walls of the artery were obtained, and the transmit zone was set to the level of the anterior vessel wall. Depth and gain settings were optimized to identify the lumen to vessel wall interface, and were kept constant during each study. Flow increase was induced by inflation of a pneumatic tourniquet placed around the forearm to 300 mm Hg, followed by cuff deflation 4–5 min later (reactive hyperemia). The artery was scanned continuously for 90 s after cuff deflation. A second resting scan was recorded 10 min later, and was followed by administration of sublingual nitroglycerin (NTG) (400 μg). The final scan was acquired 3–4 min later. These measurements were done twice and the mean of the two values was used for the vessel size of flow-mediated and NTG-induced in vegetarians and omnivores. Flow-mediated dilatation in the brachial artery following reactive hyperemia and endothelium independent dilatation following NTG administration were expressed as percentage diameter change relative to the first baseline scan. One of the authors (M-K. Gueng) performed all ultrasonographic examinations and did all measurements and was unaware of the dietary preferences of all subjects.

3. Results

The vasodilatory functions of brachial artery evaluated via ultrasonography in omnivores and vegetarians are depicted in Table 3. The ultrasonography of brachial arteries revealed no arterial thickening or plaque formation in all subjects. There was no significant difference in baseline vessel size between these two groups. In multivariate analysis, age and gender were not correlated with the degree of vasodilatation. However, both percentages of flow-mediated and NTG-induced vasodilation were significantly greater in the vegetarian group than in the omnivorous group (P < 0.001).

Table 3
Vasodilatory functions of brachial artery evaluated by ultrasonography in omnivores and vegetarians*?

<table>
<thead>
<tr>
<th></th>
<th>Omnivore (n = 20)</th>
<th>Vegetarian (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline vessel size (mm)</td>
<td>4.42 ± 0.53</td>
<td>4.21 ± 0.55</td>
<td>0.2071</td>
</tr>
<tr>
<td>Flow-mediated dilatation (%)</td>
<td>3.13 ± 1.36</td>
<td>13.78 ± 2.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NTG-induced dilatation (%)</td>
<td>13.78 ± 2.06</td>
<td>21.99 ± 2.21</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* All values are expressed as mean ± standard deviation.

Fig. 1 illustrates the correlation between percentages of both flow-mediated and NTG-induced vasodilation and years of vegetarian diets. The calculated Pearson correlation coefficient is + 0.5026 for flow-mediated (P = 0.02) and + 0.4883 for NTG-induced (P = 0.03), indicating substantial positive correlation.
The major findings of this study are: (1) the vascular dilatory responses of vegetarians assessed by flow-mediated and NTG-induced are better than those of omnivores; (2) these effects are dependent on diets alone and are independent of other known factors of atherosclerosis, such as smoking, diabetes, hypertension, hyperlipidemia, aging, etc.; and (3) the dilatory responses to flow-mediated and NTG-induced in vegetarians are well correlated with the duration being on vegetarian diets.

4.1. Flow-mediated vasodilation

Dilatation mediated by brachial-artery flow is endothelium-dependent [19] and is mediated in large part by the release of NO [20]. Endothelial dysfunction may predispose to thrombosis, leukocyte adhesion, and proliferation of smooth muscle cells in the arterial wall, and contribute to the initial stages of atherogenesis and it may occur before any clinical evidence of vascular disease [8,21,22].

Endothelial dysfunction has been demonstrated in subjects with risk factors for atherosclerosis such as family history [23], diabetes [24], hypercholesterolemia [24], cigarette smokers [25], and others such as male sex, hypertension, old age [26], and race [27]. Our study results of significant differences in the endothelial functions of asymptomatic vegetarians compared with age and sex-matched omnivores, which can not be explained by other known risk factors, indicate that diets may be a risk factor by itself for endothelial dysfunction.

4.2. Nitroglycerin-induced vasodilation

In experimental animal studies, the vasodilator responses of healthy vessels to exogenous nitric oxide (NO) are increased after endothelial denudation or acute inhibition of endothelial NO synthesis [6,7,28–30]. It has been postulated that this effect may be due to upregulation of receptors in smooth muscle cells or to a lack of partial tolerance induced by the defect of endogenously produced NO from an intact endothelium [28].

An impaired dilatory response to NTG may be due to vascular smooth muscle cell dysfunction, or due to decreased bioavailability of NO to the smooth cell, or due to mechanical forces limiting the artery's ability to relax.

The same pathologic processes that lead to endothelial dysfunction in adults (such as hypercholesterolemia or smoking) may also be affecting smooth muscle function due to decreased bioavailability of NTG/NO to the vascular smooth muscle cell [12]. The overproduction of free radicals in the vessel wall associated with hypercholesterolemia may combine with endothelial NO, forming peroxynitrite, hence decreasing the bioavailability of NO to the smooth muscle cell and functional changes within the smooth muscle cell such as decreased activity of intracellular guanylate cyclase, cyclic GMP or calcium-dependent relaxation may also alter the reactivity to NTG [12]. Vegetarian’s better dilatory response to nitroglycerin in our study suggests that long term vegetarian diets may have a protective effect upon vascular smooth muscle independent of other risk factors.

4.3. Mechanisms

We know from nutritional surveys in Taiwan, vegetarians consumed fewer calories, more carbohydrate (63% of calories in men, 58% in women), less protein (12% of calories) and fat (25% for men, 30% for women) [15]. The polyunsaturated-saturated (P/S) and polyunsaturated/monounsaturated (P/M) fatty acid ratios in this Taiwanese vegetarian diets (P/S ≈ 3.4, P/M ≈ 2.5) were high [15,16].

The vascular dilatory responses of vegetarians, both flow-mediated and NTG-induced, are better than those of omnivores and it remains unclear as to which component of vegetarian diets causes these effects. Is it less protein? less fat? the abundance of foods that contains arginine which is a precursor of nitric oxide? or, simply the lack of deleterious effect of meat? It remains to be determined.

In summary, significant differences of both flow-mediated (endothelium-dependent) vasodilatation and exogenous NTG-induced (endothelium-independent) were noted between asymptomatic long-term vegetarians and
sex, age-matched omnivores. The degree of vasodilation correlated positively with years being on vegetarian diets. These cannot be explained on the basis of known risk factors such as hypertension, diabetes mellitus, hypercholesterolemia, cigarette smoking, gender, aging, etc. Thus, our study results, although only a small, cross-sectional observation, suggest that a vegetarian diet, by itself, may have a significant vascular protective effect and may help to account for the lower incidence of atherosclerosis and cardiovascular mortality seen in vegetarians but the exact mechanisms remain unclear. A larger scale, longitudinal comparison of vasodilatory functions between vegetarians and omnivores in various stages of atherosclerosis may shed light on the mechanisms of vascular protective effect of vegetarian diets and help in our understanding of atherogenesis.

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