Effects of Green-Leafy Vegetable Intake on Postprandial Glycemic and Lipidemic Responses and α-Tocopherol Concentration in Normal Weight and Obese Men

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Summary Vegetable consumption has been encouraged as a component of nutritional education for obese and insulin-resistant patients. However, the benefits of vegetable intake in a therapeutic diet on postprandial glycemic and lipidemic responses have not been clarified. We studied the effects of the intake of spinach, a green-leafy vegetable rich in dietary fiber and α-tocopherol, with a fat-rich meal on postprandial glycemic and lipidemic changes.

Subjects. Fourteen normal weight subjects (BMI<25) and 10 obese subjects (BMI≥25), 20 to 35 y of age and not receiving treatment for medical conditions or using supplements, participated in this study. Written informed consent, the protocol of which had been approved by the Japan Women’s University ethics committee, adhered closely to the 1995 Declaration of
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Table 1. Food composition and nutrient and energy contents of three test meals.

<table>
<thead>
<tr>
<th>Foods</th>
<th>Bread meal (B)</th>
<th>Bread &amp; butter meal (BB)</th>
<th>Bread, butter &amp; spinach meal (BBS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread (g)</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>Butter (g)</td>
<td>—</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Boiled spinach (g)</td>
<td>—</td>
<td>—</td>
<td>75</td>
</tr>
<tr>
<td>Hot water (mL)</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Nutrients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>449</td>
<td>635</td>
<td>654</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>15.8</td>
<td>16</td>
<td>17.9</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>7.5</td>
<td>27.7</td>
<td>28.1</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>0</td>
<td>52.5</td>
<td>52.5</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>75.5</td>
<td>75.5</td>
<td>75.8</td>
</tr>
<tr>
<td>Soluble dietary fiber (g)</td>
<td>0.7</td>
<td>0.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Insoluble dietary fiber (g)</td>
<td>3.2</td>
<td>3.2</td>
<td>5.5</td>
</tr>
<tr>
<td>α-Tocopherol (mg)</td>
<td>0.9</td>
<td>1.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Helsinki (revised in Edinburgh in 2000), was obtained from all participants prior to their inclusion in this study.

Test meals. All subjects consumed three types of test meals given at random: bread and butter (BB), spinach with BB meal (BBS), and a control meal of bread alone (B) (Table 1). The B meal contained 170 g of white bread, the BB meal contained 25 g of butter in addition to 170 g of white bread, and the BBS meal contained 75 g of boiled spinach (100 g of raw spinach boiled for 30 s, soaked in cold water for 5 min and then cut into 5-cm-long strips), in addition to the BB meal. Spinach, which is high in dietary fiber and α-tocopherol, is a popular leafy green vegetable in Japan. One hundred grams of this vegetable is the recommended volume for consumption in one meal for Japanese diabetic patients (20). The calculated nutrient composition based on standard tables of food components in Japan (5th edition) is shown in Table 1. All meals contained about the same amount of carbohydrate. The energy amounts derived from fat in the B, BB, and BBS meals were 15.0, 39.3 and 38.7%, respectively. In the BBS meal, the total volume of dietary fiber and α-tocopherol were about one-third and one-half, respectively, of the daily dietary recommendations (21).

Protocol. The subjects were instructed to finish supper before 21:00 on the day before the test, and to arrive at Japan Women’s University by 08:30 on the test date. Anthropometric measurements and fasting blood collection were thus performed after an overnight fast. The subjects consumed the test meal with 200 mL of hot water within 20 min. Blood samples were drawn at 30, 60, 120, 180 and 240 min after finishing the meal. During the test, the subjects remained seated. The washout period between two tests was 1 wk. The subjects were asked not to alter their usual food intake patterns or physical activity levels during the study period.

Measurements. At all time points, blood was collected into two tubes: one for serum and one for plasma glucose analysis (containing sodium fluoride and potassium oxalate). Plasma and serum were separated from whole blood by centrifugation (1,500 × g for 15 min) at 4˚C. Plasma glucose was measured using a TOSOH HLC-723GHBV auto-analyzer, aspartate aminotransferase and alanine aminotransferase by the UV method, γ-glutamyl transpeptidase by a colorimetric method, and total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol and triglycerides by enzymatic methods using a HITACHI 7170S autoanalyzer in the laboratory at Saitama Social Insurance Hospital. Serum insulin was measured by radioimmunoassay at Bio Medical Laboratories, Inc., Tokyo. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated employing the following formula: fasting blood glucose (mmol/L)/22.5. The following parameters were measured in fasting and postprandial samples: plasma glucose, serum insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, and α-tocopherol.

α-Tocopherol measurement. Serum α-tocopherol was measured using a modification of the Abe and Katsui method with high performance liquid chromatography (22), as described previously (23). The chromatographic conditions used with the fluorometric detector, SHIMADZU SCL-10A (Shimadzu Co., Kyoto, Japan), were as follows: column, shim-pack CLC-NH2 (4.6 × 250 mm) (Shimadzu Co.); mobile phase, isopropyl alcohol/n-hexan (2 : 100); flow rate, 1.0 mL/min; and injection volume, 10 μL. Results were accepted as valid if the CV for a control sample of a known concentration was below 5%.

Calculations and statistical analysis. The serum α-tocopherol concentration was corrected employing the sum of serum triglycerides and the total cholesterol concentration as α-tocopherol/lipids according to the method used in a prior study (24).

The incremental areas under the curve (IAUC) were calculated for plasma glucose, serum insulin and triglycerides, and total cholesterol, LDL-cholesterol and HDL-cholesterol.
α-tocopherol/lipid concentrations were calculated as the decremental (dAUC) values from baseline using the 4-h trapezoid rule. Statistical analyses were performed using SPSS for Windows (version 16J, SPSS Japan Inc., Tokyo, Japan). Data were expressed as means±standard error (SE). Normality was assessed by the Kolmogorov-Smirnov test. The Mann-Whitney U-test at p<0.05 was used to identify differences between healthy and obese subjects. Multiple comparisons between mean values were made by analysis of variance (repeated measures), followed by the Mann-Whitney U-test with Bonferroni’s correction. p<0.05 was considered to indicate a statistically significant difference.

RESULTS

Subject characteristics in fasted state

Physical characteristics and the means of biochemical parameters in the fasted state for the three test meal loading days are shown in Table 2. Mean BMI, waist circumference, serum insulin, HOMA-IR, and triglyceride, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase and α-tocopherol concentrations were higher, while the HDL-cholesterol concentration was lower, in obese than in normal subjects. Postprandial changes in glycemic and lipid parameters and α-tocopherol concentration and differences in AUC among the three test meals during a 240-min period (Fig. 1, Table 3)

Figure 1 shows postprandial plasma glucose, triglyceride, LDL-cholesterol and α-tocopherol changes from fasting to 240 min. IAUC and dAUC for postprandial glycemic and lipid concentrations are shown in Table 3. Plasma glucose and serum insulin

In normal subjects, the change in plasma glucose at 30 min after a BB meal was 0.9±0.3 mmol/L, which was less than the 1.9±0.2 mmol/L after the B meal (p<0.05), and the IAUC for plasma glucose with the BB meal was thus lower than that with the B meal (p<0.05). However, the subjects showed no differences in postprandial glucose changes after consuming the BB versus the BBS meal.

In obese subjects, no significant differences were observed in postprandial glucose levels among the three meals.

The serum insulin concentration increased, peaking at 30 min after meal consumption, with no differences at any time during the 240 min, among the meals in either normal or obese subjects.

Plasma glucose-IAUC and insulin-IAUC after the BB and BBS meals were higher in obese than in normal subjects.

Triglyceride

The postprandial triglyceride concentration did not change after B meal consumption in normal subjects. Triglyceride levels were higher after the BB and BBS meals, from 30 to 240 min, as compared with after B meal consumption (p<0.05). IAUC values for triglycerides with the BB and BBS meals were greater than that with the B meal (p<0.05) in normal subjects.

On the other hand, the postprandial triglyceride level continued to rise after the B meal, with a mean increment of 0.44±0.12 mmol/L at 240 min in obese subjects. Furthermore, a marked postprandial triglyceride increase was observed after the BB and BBS meals. At 120 min after BBS meal consumption especially, the mean increment of 0.71±0.09 mmol/L was significantly higher than that following the B meal (p<0.05). IAUC for triglycerides with the BBS meal did not differ from that with the BB meal, but was greater than that with the B meal (p<0.05) in obese subjects.

Triglyceride IAUC values after the B and BBS meals were higher in obese than in normal subjects (p<0.01).

LDL-cholesterol

The LDL-cholesterol concentration decreased after
Fig. 1. Mean postprandial changes in plasma glucose, triglyceride, LDL-cholesterol and α-tocopherol/lipid during 240 min after ingestion of 3 test meals. Data are presented as means±SE. B, bread meal; BB, bread and butter meal; BBS, bread, butter and spinach meal. * Significant difference from B meal at \( p<0.05 \); ‡ significant difference from BB meal at \( p<0.05 \).
all three meals. In normal subjects, the LDL-cholesterol concentration decreased by 0.10±0.03 mmol/L at 30 min and this was maintained throughout the 240 min after B meal consumption. Even more marked decreases of 0.23±0.06 mmol/L after the BB meal and 0.18±0.03 mmol/L after the BBS meal, in contrast to the B meal, were seen at 120 min (p<0.05).

In obese subjects, the decrease in LDL-cholesterol was greater, 0.21±0.02 mmol/L at 120 min after the B meal. After BB meal consumption, LDL-cholesterol continued to fall, dropping by 0.40±0.05 mmol/L at 120 min, i.e. a larger decrease than with the B meal (p<0.05), then increased thereafter. These changes resulted in a much greater dAUC for the BB meal than for the B meal (p<0.05). As for the BBS meal, the decrease was almost the same as that after the B meal at 60 min, then further decreases were seen at 120 and 180 min as compared to after the BB meal.

LDL-cholesterol dAUC, shown as negative values, were larger after all three meals in obese than in normal subjects (p<0.05).

**HDL-cholesterol**

The HDL-cholesterol concentration decreased after all three meals. HDL-cholesterol decreased by 0.04±0.01 mmol/L at 30 min and this was maintained throughout the 240-min period after B meal consumption in both groups, with no differences among the three meals in either normal or obese subjects.

**α-Tocopherol/lipid**

The α-tocopherol/lipid concentration tended to decrease after the consumptions of all three meals, but there were no differences among the three meals in normal subjects.

However, in obese subjects, an immediate α-tocopherol/lipid decrease was observed after BB meal consumption. The decrease was smaller with the BBS than with the BB meal at 120 min (p<0.05), resulting in a tendency for a smaller α-tocopherol/lipid dAUC with the BBS than with the BB meal (p=0.066).

**DISCUSSION**

We confirmed a restricted postprandial glucose increase with butter consumption as compared to only bread intake in normal weight subjects, as reported previously (19). However, we found that consuming 25 g of butter with 75 g of carbohydrate from white bread did not exert a lowering effect on the postprandial glucose response in obese subjects. Fat is generally considered to reduce glycemic responses by delaying gastric emptying, and thus reduces the rate of glucose absorption and the rise in postprandial insulin partially in relation to incretin (25–27). However, an impaired incretin response after meal consumption has been reported to be associated with insulin resistance (28–31). Our results in obese subjects are consistent with those of prior reports.

Regarding postprandial changes in serum lipid concentrations, there was a large triglyceride increase after the B meal in obese but not in normal subjects. This supports the view that accelerated triglyceride synthesis and impaired ability to suppress very low density lipo-

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**Table 3. Incremental and decremental areas under the curve of mean postprandial parameters 240 min after test meal.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal (n=14)</th>
<th>Obese (n=10)</th>
<th>p value Normal vs Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAUC B</td>
<td>135±26</td>
<td>229±40</td>
<td>0.096</td>
</tr>
<tr>
<td>Plasma glucose (mmol-min/L) B</td>
<td>71±18*</td>
<td>207±29</td>
<td>0.001</td>
</tr>
<tr>
<td>IAUC B</td>
<td>91±33</td>
<td>268±50</td>
<td>0.003</td>
</tr>
<tr>
<td>Serum insulin (pmol-min/L) BBS</td>
<td>36.492±4.619</td>
<td>45.445±9.980</td>
<td>0.546</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol-min/L) B</td>
<td>28.829±3.494</td>
<td>48.410±7.465</td>
<td>0.031</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol-min/L) BBS</td>
<td>32.687±4.051</td>
<td>57.650±5.259</td>
<td>0.002</td>
</tr>
<tr>
<td>dAUC B</td>
<td>12±4</td>
<td>69±18</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglyceride (mmol-min/L) BBS</td>
<td>76±15*</td>
<td>114±23</td>
<td>0.172</td>
</tr>
<tr>
<td>dAUC B</td>
<td>30±6</td>
<td>−20±4</td>
<td>0.212</td>
</tr>
<tr>
<td>Total cholesterol (mmol-min/L)B</td>
<td>−23±5</td>
<td>−37±7</td>
<td>0.122</td>
</tr>
<tr>
<td>dAUC B</td>
<td>−22±5</td>
<td>−38±5</td>
<td>0.009</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol-min/L) B</td>
<td>−41±9</td>
<td>−76±10*</td>
<td>0.022</td>
</tr>
<tr>
<td>dAUC B</td>
<td>−33±5</td>
<td>−59±6*</td>
<td>0.007</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol-min/L) B</td>
<td>−10±2</td>
<td>−6±2</td>
<td>0.259</td>
</tr>
<tr>
<td>dAUC B</td>
<td>−10±2</td>
<td>−12±3</td>
<td>0.931</td>
</tr>
<tr>
<td>α-Tocopherol/lipid (μmol-min/mmol) BBS</td>
<td>−12±3</td>
<td>−7±2</td>
<td>0.172</td>
</tr>
<tr>
<td>dAUC B</td>
<td>−60±15</td>
<td>−45±13</td>
<td>0.709</td>
</tr>
<tr>
<td>α-Tocopherol/lipid (μmol/min/mmol) BBS</td>
<td>−89±19</td>
<td>−135±30</td>
<td>0.172</td>
</tr>
<tr>
<td>dAUC B</td>
<td>−73±19</td>
<td>−56±12</td>
<td>0.285</td>
</tr>
</tbody>
</table>

Data are means±SE.

* Significant difference from B meal at p<0.05.

B, bread meal; BB, bread & butter meal; BBS, bread, butter & spinach meal; AUC, area under curve.
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protein-triglyceride secretion lead to insulin resistance (32). After BB meal consumption, we also observed an increase in triglycerides in both normal and, more significantly, in obese subjects. The postprandial remnant dyslipidemia which results from insulin resistance in visceral obesity has been reported with decreased lipoprotein lipase activity (33) and lipoprotein receptor expression (34). From the clinical viewpoint of suppressing the postprandial triglyceride increase, it should be emphasized that fat intake with carbohydrate cannot be recommended as a means of reducing postprandial plasma glucose spikes in obese subjects.

The main objective of this study was to assess the effects of intake of a green leafy vegetable, rich in fiber and α-tocopherol, on postprandial hyperglycemic and hyperlipidemic changes with a fat-added meal in obese subjects. Despite containing 6.6 g of dietary fiber in total, which is one-third of the daily recommendation, spinach did not suppress the postprandial glucose or triglyceride increase in either normal or obese subjects. There are few studies on the effects of fiber from vegetables on postprandial status. The only study showing a postprandial glucose reduction by green leafy vegetables was reported by Gustafsson et al. who described feeding a huge amount of spinach (250 g) contained in a mixed meal to healthy subjects (35). Furthermore, soluble fiber has been considered most likely to be therapeutically useful in modifying postprandial hyperglycemia through a reduction in the rate of gastric emptying and small intestinal absorption (36, 37). However, despite ingestion of the recommended serving size of vegetables for one meal, containing 6.6 g of dietary fiber in total but only 1.1 g of soluble fiber, in the form of boiled spinach, there was no reduction in the postprandial glucose response in this study. These results suggest that vegetable intake cannot be expected to suppress postprandial glycemic or lipidemic responses with consumption of a dish of the currently recommended size.

An additional interesting observation was that the reductions in LDL-cholesterol and α-tocopherol/lipid concentrations were smaller after the BBS meal than after the BB meal in obese subjects. The marked decrease in the LDL-cholesterol concentration in our obese subjects as compared to normal subjects is consistent with the reported findings that bile acid synthesis and secretion are accelerated in type 2 diabetic patients (38, 39).

Fat intake stimulates bile acid secretion with gallbladder contraction, which accelerates the transport of esterified cholesterol from LDL to triglyceride-rich lipoprotein mainly via chylomicrons and circulating LDL inverse transport to the liver, resulting in the lowering of serum LDL-cholesterol (40). In this study, the postprandial triglyceride increase was nearly the same after the BB and BBS meals. Thus, transition differences among lipoproteins would not explain the smaller LDL-cholesterol reduction observed. Postprandial LDL-cholesterol levels reportedly decrease with the amount of ingested fat (40). The possible explanations for the smaller LDL-cholesterol reduction with the BBS meal include co-consumption of spinach with butter possibly delaying the passage of butter from the stomach into the small intestine, thereby diminishing bile acid secretion.

As for absorption of fat-soluble nutrients, Narushima et al. reported that cholesterol and α-tocopherol are absorbed via the Nieman-pick C1-like 1 receptor (41), which suggests that these nutrients compete for transport. In an animal study, it was shown that enterocytes use two pathways for vitamin E absorption and that the major pathway of α-tocopherol absorption is via chylomicrons (42). Furthermore, in a human study with fat-rich meal feeding, α-tocopherol in LDL and HDL decreased postprandially, concomitantly with a rise in triglyceride-rich lipoprotein α-tocopherol in healthy subjects (43), and plasma concentration of α-tocopherol was reported to be increased at 2 h after supplementation (44). These reports support the difference of α-tocopherol/lipid concentration at 120 min after the BB meal and after the BBS meal in this study. A major reason for the observed smaller reduction in the α-tocopherol/lipid concentration after the BBS meal as compared with after the BB meal was that the ingested spinach delayed passage of butter from the stomach into the small intestine with reduced bile acid secretion, as mentioned above, which might have promoted retention of endogenous α-tocopherol, in addition to the newly absorbed α-tocopherol from the spinach.

On the other hand, Cardona et al. reported that a high-fat meal increases postprandial triglyceride and generates strong oxidative stress, because circulating antioxidant defences are decreased (45). Thus, exogenous antioxidants present in LDL are considered to be the first line of defence against oxidation (46, 47).

There have been few studies on postprandial serum α-tocopherol changes associated with natural vegetable consumption together with fat. In this study, after BBS meal consumption, decreases in LDL cholesterol and α-tocopherol/lipid were smaller than after BB meal consumption, suggesting that α-tocopherol might have transport priority in obese subjects. Obese subjects were reported to have higher circulating oxidized LDL levels, with increased susceptibility to oxidation of plasma LDL than normal subjects (48). The high triglyceride/HDL-cholesterol ratios in the obese subjects in this study suggested that they had high levels of small dense LDL (49).

Thus, obese patients might require more antioxidants for protection against oxidative stress. This raises the possibility that the difference in postprandial α-tocopherol responses after the BBS meal between normal and obese subjects correlates with increased postprandial triglyceride and anti-oxidant capacity. While nuts and vegetable oils are also rich in α-tocopherol, consumption of these foods leads to excessive energy intake from fat. The results of this study indicate that obese subjects should increase their α-tocopherol intake, to protect against oxidative stress, not with energy-dense foods but rather with low energy dishes such as green leafy vegetables.

This study has limitations. As we did not study postprandial changes with bread and spinach consumption
without butter, the benefits of vegetable consumption with only carbohydrate could not be ascertained. We did not measure parameters of oxidative status, because butter is rich in saturated fatty acids. If oils rich in polyunsaturated fatty acids were consumed, the α-tocopherol requirement would be further increased. The effects of green leafy vegetable consumption with plant and/or fish oils on the postprandial state, including oxidative stress, warrant further study.

We conclude that fat-added meals cause postprandial hyperglycemic and hypertriglyceridemic changes in obese subjects. Consumption of green leafy vegetables such as spinach, with a fat-rich meal, is of benefit for effectively supplying α-tocopherol in obese subjects. However, care should be taken in nutritional education, as preventive effects against postprandial hyperglycemia and hypertriglyceridemia cannot be expected with consumption of regular-sized vegetable portions by obese subjects.

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