Four week supplementation with mixed fruit and vegetable juice concentrates increased protective serum antioxidants and folate and decreased plasma homocysteine in Japanese subjects

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Fruit and vegetable consumption has been inversely associated with the risk of chronic diseases including cancer and cardiovascular disease, with the beneficial effects attributed to a variety of protective antioxidants, carotenoids and phytonutrients. The objective of the present study was to determine the effect of supplementation with dehydrated concentrates from mixed fruit and vegetable juices (Juice Plus+) on serum antioxidant and folate status, plasma homocysteine levels and markers for oxidative stress and DNA damage. Japanese subjects (n=60; age 27.8 yrs; BMI 22.1) were recruited to participate in a double-blind placebo controlled study and were randomized into 2 groups of 30, matched for sex, age, BMI and smoking status (39 males, 22 smokers; 21 females, 13 smokers). Subjects were given encapsulated supplements containing mixed fruit and vegetable juice concentrates or a matching placebo for 28 days, with blood and urine samples collected at baseline, day 14 and day 28 for analytical testing. Compared with the placebo, 28 day supplementation significantly increased the concentration of serum beta-carotene 528% (p<0.0001), lycopene 80.2% (p<0.0005), and alpha tocopherol 39.5% (p<0.0001). Serum folate increased 174.3% (p<0.0001) and correlated with a decrease in plasma homocysteine of -19.9% (p<0.03). Compared with baseline, measures of oxidative stress decreased with serum lipid peroxides declining -10.5% (p<0.02) and urine 8OHdG decreasing -21.1% (p<0.02). Evaluation of data from smokers only (n=17) after 28 days of active supplementation showed comparable changes. Conclusion: In the absence of dietary modification, supplementation with the fruit and vegetable juice concentrate capsules proved to be a highly bioavailable source of phytonutrients. Important antioxidants were elevated to desirable levels associated with decreased risk of disease while markers of oxidative stress were reduced, and folate status improved with a concomitant decrease in homocysteine, and these benefits occurred to a similar extent in smokers when compared to non-smokers.

Key Words: fruits, vegetables, antioxidants, folate, homocysteine, carotenoids, smokers

INTRODUCTION
Epidemiological evidence suggests that a high level of fruit and vegetable consumption can reduce the risk of developing diseases, particularly cancers, cardiovascular disease (CVD), metabolic disorders and stroke.¹⁻⁵ There is evidence in Japanese populations, as well as for other nationalities, that the frequent consumption of fruits and vegetables is associated with a reduced overall risk of cancer mortality.⁶⁻⁷ Moreover, high levels of antioxidant micronutrients such as carotenoids and lycopene, found in abundance in fruits and vegetables, have been shown to reduce cancer mortality rates in Japanese.⁸⁻⁹ Additionally, smokers in Japan as well as elsewhere¹⁰ have been reported to have lower dietary intake of fruits and vegetables and lower serum concentrations of dietary antioxidants.

This inverse association between fruit and vegetable intake and chronic disease appears to hold true across different geographical locations and in populations that differ in lifestyle, gender and age – a paradigm prompting health authorities to recommend increased consumption of fruits and vegetables and to implement this public health directive in many countries.¹²⁻¹⁷

One mechanism by which fruits and vegetables exert a protective effect appears to be related to a variety of bioactive compounds which can reduce oxidative stress.¹⁸⁻²² Fruits and vegetables contain many antioxidants including carotenoids, tocopherols, ascorbic acid, and polyphenols...
which are able to quench reactive oxidants (free radicals) and reduce oxidative damage to cell structures, and cellular DNA.\textsuperscript{23-25} Another mechanism of protection may involve activation of genes which code for antioxidant enzymes and/or silencing genes which may promote oxidative stress.\textsuperscript{26-29} Dietary antioxidants may therefore mitigate against oxidative damage by directly inactivating reactive oxidants and by modulating gene expression contributing to oxidative stress. Additional protection provided by fruit and vegetable consumption may be afforded by improved folate status resulting in reduced serum homocysteine levels – a recognized risk factor for heart disease and stroke.\textsuperscript{30-32} Furthermore, folate has been shown to be essential for a variety of methylation reactions which insure normal DNA replication, transcription, and cell division and explain how depressed folate status may increase the risk for cancer and neural tube defects.\textsuperscript{33,34} Folate as well as antioxidants may also contribute to vascular health by improving endothelial function.\textsuperscript{35,36}

Smoking, in addition to being a well recognized risk factor for cancer and CVD, has been correlated with a lower consumption of fruits and vegetables among smokers compared to non-smokers.\textsuperscript{37,38} Smoking is associated with increased oxidative stress,\textsuperscript{39} and smokers and passive smokers both have a significantly lower plasma antioxidant status than do unexposed non-smokers, independent of dietary antioxidant intakes.\textsuperscript{37,38} Poor antioxidants status in smokers is reflected in measures of oxidative stress as measured by increased serum 8OHdG\textsuperscript{39} and plasma malondialdehyde.\textsuperscript{40}

In spite of the recognized protective health benefits attributable to fruits and vegetables and the recommendations of health organizations that individuals increase their daily intake, consumption has hardly changed over the past decade.\textsuperscript{41} One approach to improving compliance may be to provide important phytonutrients derived from fruits and vegetables in the form of a supplement. Such preparations have been developed using dehydrated concentrates from mixed fruit and vegetable juices, and the previous studies using these mixtures have demonstrated bioavailability of bioactive antioxidants,\textsuperscript{42,43} improvement in measures of immune function,\textsuperscript{44} reduction in DNA damage\textsuperscript{45} and improved endothelial function.\textsuperscript{46}

The aim of the present study was to determine the bioavailability of important antioxidants from a mixed concentrate of fruit and vegetable juice powders compared to placebo capsules, and the effects on oxidative stress, folate status, and homocysteine in both smokers and non-smokers. We tested the hypothesis that increased phytonutrient intake from fruits and vegetables when provided in the form of a supplement, could alter measurable risk factors for disease in healthy, young Japanese subjects, particularly in smokers, in absence of other dietary modification.

**MATERIALS AND METHODS**

**Study design**

Subjects participated in a randomized, double-blind, placebo-controlled, parallel-group trial, consisting of 28 days’ treatment with a dietary supplement (Juice Plus+®; NSA, Inc., Memphis, TN, USA) or physically identical placebo. Subjects were instructed to take their capsules twice daily with meals. The active capsules contained primarily juice powder concentrate from apple, orange, pineapple, papaya, cranberry, acerola cherry, peach, carrot, parsley, beetroot, broccoli, cabbage, spinach, tomato, kale, along with barley and oat bran. These fruit and vegetable capsules provided approximately 420 µg folate, 234 mg vitamin C, 32 mg vitamin E, 7.5 mg beta-carotene equivalents from mixed carotenoids and 160 mg bioflavonoids per day. Subjects were asked to maintain their normal dietary and exercise patterns and not to take any additional vitamin or herbal supplements during the trial.

**Subjects**

Subjects were recruited from a database of individuals living and/or working in Tokyo, Japan. All potential trial participants were interviewed by using a short questionnaire that sought information about their general health, family history, use of medication or nutritional supplements and smoking status. Trial participants satisfied the following inclusion criteria: age of 18–50 years, not taking nutritional supplements, not taking medication for a chronic disease, a body mass index (BMI) within normal limits (between 20 and 24 kg/m²), not suffering from a chronic disease, without a history of metabolic disease and willing to give written informed consent to take part in the trial. The following exclusion criteria also applied: pregnant or lactating women, those judged to be unsuitable to take part in the study by the responsible doctor, subjects eating more than average levels of fruit and vegetables and subjects who reported high levels of physical activity. A local independent Institutional Review Board approved the study protocol. All subjects participating in this trial gave their written, informed consent.

**Screening evaluations**

After the initial screening of 140 individuals, 100 subjects were selected for further testing. Exercise levels, smoking status and quantity, evaluation of diet, blood pressure and pulse, and blood chemistries of the subjects were investigated during the secondary screening phase. Dietary intake of each subject was determined by a 3-day food recall during screening and at 4 weeks. A food diary was not used as it may influence food choices during the study. A dietary analysis was performed based on the foods which the subjects reported to have eaten, and software (Eiyan Sodan, Olympus Optics Systems) was used to calculate nutritional values. Software was based on Standard Tables of Food Composition in Japan, Fifth Revised Edition published by the Japanese Ministry of Health, Labor and Welfare.\textsuperscript{47}

Blood and urine samples were taken 2 weeks prior to the start of the study to evaluate standard hematologies and chemistries and subjects with abnormalities in one or more measurements were excluded. Serum β-carotene was also measured as a surrogate marker for high consumption of fruit and vegetables, with subjects >0.300 µg/mL excluded.

After secondary screening, 60 qualified subjects were randomised by a biostatistician into two comparable
The table below represents the Japan study baseline characteristics:

### Table 1. Japan study baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>All Subjects</th>
<th>Male (Active)</th>
<th>Male (Placebo)</th>
<th>Female (Active)</th>
<th>Female (Placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>60</td>
<td>20</td>
<td>19</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Age, y</td>
<td>27.8 ± 0.91</td>
<td>27.9 ± 1.55</td>
<td>25.2 ± 1.17</td>
<td>29.2 ± 2.42</td>
<td>30.9 ± 2.64</td>
</tr>
<tr>
<td>Smokers, n</td>
<td>35</td>
<td>12</td>
<td>10</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>(Pack Years)</td>
<td>6.62 ± 1.25</td>
<td>7.05 ± 2.64</td>
<td>5.35 ± 1.44</td>
<td>1.78 ± 0.60</td>
<td>9.95 ± 4.03</td>
</tr>
<tr>
<td>Height, cm</td>
<td>169 ± 1.1</td>
<td>172 ± 0.9</td>
<td>175 ± 1.6</td>
<td>162 ± 1.5</td>
<td>160 ± 2.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.7 ± 0.95</td>
<td>66.0 ± 0.97</td>
<td>69.1 ± 1.25</td>
<td>56.1 ± 1.73</td>
<td>56.9 ± 1.71</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.1 ± 0.16</td>
<td>22.2 ± 0.24</td>
<td>22.4 ± 0.27</td>
<td>21.1 ± 0.47</td>
<td>22.3 ± 0.36</td>
</tr>
<tr>
<td>Energy, kCal/d</td>
<td>1687 ± 53.3</td>
<td>1649 ± 102</td>
<td>1747 ± 110</td>
<td>1700 ± 114</td>
<td>1641 ± 82.4</td>
</tr>
<tr>
<td>Protein, g</td>
<td>58.5 ± 2.2</td>
<td>56.3 ± 4.2</td>
<td>62.5 ± 5.0</td>
<td>55.9 ± 3.5</td>
<td>58.1 ± 3.0</td>
</tr>
<tr>
<td>Fat, g</td>
<td>62.3 ± 2.8</td>
<td>56.2 ± 3.9</td>
<td>64.6 ± 5.8</td>
<td>66.6 ± 7.8</td>
<td>65.7 ± 5.6</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>208 ± 6.5</td>
<td>212 ± 13.2</td>
<td>214 ± 13.3</td>
<td>208 ± 9.9</td>
<td>194 ± 9.4</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>76.2 ± 26.3</td>
<td>59.1 ± 16.4</td>
<td>123 ± 81.4</td>
<td>56.9 ± 7.8</td>
<td>44.1 ± 6.9</td>
</tr>
<tr>
<td>Vitamin E, mg</td>
<td>5.96 ± 0.29</td>
<td>5.56 ± 0.56</td>
<td>6.02 ± 0.55</td>
<td>6.44 ± 0.78</td>
<td>6.15 ± 0.47</td>
</tr>
<tr>
<td>Folate, μg</td>
<td>185 ± 14</td>
<td>164 ± 16</td>
<td>194 ± 36</td>
<td>232 ± 34</td>
<td>164 ± 14</td>
</tr>
<tr>
<td>Retinol Equiv, μg</td>
<td>398 ± 26</td>
<td>346 ± 34</td>
<td>373 ± 53</td>
<td>506 ± 76</td>
<td>433 ± 61</td>
</tr>
<tr>
<td>Carotene, μg</td>
<td>1417 ± 114</td>
<td>1223 ± 165</td>
<td>1300 ± 188</td>
<td>1706 ± 314</td>
<td>1653 ± 310</td>
</tr>
</tbody>
</table>

†Values are mean ± SEM

Effects of fruit and vegetable juice concentrates on antioxidants folate and homocysteine

Groups of 30 (Groups A and B) based on sex, age, BMI, smoking status and screening serum β-carotene levels. Active and placebo groups were designated based on a fair coin flip, which blinded the biostatistician. Numbers 1–60 were then randomly assigned to blind the subjects and investigators.

**Blood Samples**

At 0, 14 and 28 days, blood samples were collected for biochemical evaluations. Collection of blood samples was as follows: 15 mL blood serum, divided into three 5 mL aliquots and frozen at –80°C; 7 mL blood plasma (EDTA), divided into two 3.5 mL aliquots and frozen at –80°C.

**Biochemical analyses**

Before analysis, all frozen samples were thawed and centrifuged at 1000 x g for 10 min at 4°C, to sediment any precipitated fibrin. Samples were analysed in batches at the end of the study.

Serum β-carotene, α-carotene, α-tocopherol, lycopene, lutein + zeaxanthin, β-cryptoxanthin, retinol, were measured by HPLC (Hitachi HPLC System D-7000, Hitachi High-Technologies Corporation, Tokyo, Japan) with UV detection (L-7455, Hitachi High-Technologies Corporation, Tokyo, Japan) and fluorescence detection (L-7480, Hitachi High-Technologies Corporation, Tokyo, Japan).

Serum folate concentrations were determined by the Chemiluminescent immunoasay (ACS: 180 PLUS Automated Chemiluminescence System, Bayer Medical Ltd., Tokyo, Japan).

In this study, plasma homocysteine was measured by HPLC (LC-9A, Shimadzu Corp., Kyoto, Japan) with fluorescence detection (F-1080, Hitachi Ltd., Tokyo, Japan).

Serum lipid peroxides were determined by the Yagi alternative method (Hemoglobin methylene blue method) and analysed in an automated system (7020 Clinical Analyzer, Hitachi High-Technologies Corporation, Tokyo, Japan).

Serum vitamin C was measured by a colorimetric method and analysed in an automated system (7020 Clinical Analyzer, Hitachi High-Technologies Corporation, Tokyo, Japan).

Urine 8-OHdG concentrations were assayed by the ELISA method using the New 8-OHdG Check (ELISA kit for 8-hydroxy-2′-deoxyguanosine: Japan Institute for the Control of Aging, Fukuoka, Japan) with quantitative reaction levels measured by a microplate reader (Microplate EIA Autoanalyzer AP-960 system, Kyowa Medex, Tokyo, Japan).

**Statistical analysis**

The data were analyzed using SAS statistical software (SAS Institute, Inc., Cary, NC, USA). Proc Mixed procedure was used to analyze repeated measures. Adjusted least square means and their standard errors were calculated at individual time point (0, 14 and 28 days) within groups, and means comparison between groups at individual time point.

**RESULTS**

Sixty subjects were selected for the study and randomly assigned to placebo (n=30) and active (n=30) groups. All subjects completed the study, with their baseline data presented in Table 1. The degree of compliance was 100% based on the absence of any returned capsules and exit interviews with each subject. No significant baseline differences were noted between subjects in the active group compared to the placebo group. Also, smokers and non-smokers did not differ in nutrient intake or other measurements (data not shown).

The high degree of compliance was reflected in a significant increase in serum β-carotene after active supplementation compared to baseline (528%; p<0.0001; Table 2), and compared to placebo (p=0.0005). Similarly, serum α-tocopherol and serum lycopene increased significantly (Table 2). Vitamin C increased significantly at 14 days (p<0.001) but not at 28 days (Table 2). Serum lutein/zeaxanthin, β-cryptoxanthin and retinol concentrations did not change over the course of this investigation in either study group.
### Table 2. Effects of supplementation with a placebo or mixed fruit and vegetable concentrates (active) on various analytes from serum^1^, plasma^2^ and urine^3^ measured at baseline, 14 days and 28 days

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 14 Day 28 Day Change %</td>
<td>Baseline 14 Day 28 Day Change %</td>
</tr>
<tr>
<td>α-tocopherol (μg/mL)^1</td>
<td>11.2 ±0.55 11.5 ±0.65 0.260 11.7 ±0.76 0.42</td>
<td>11.5 ±0.55 14.6 ±0.65 3.07 ^e 26.6 16.1 ±0.76 4.56 ^e</td>
</tr>
<tr>
<td>Vitamin C (μg/mL)^1</td>
<td>8.47 ±0.45 6.98 ±0.53 -1.49 ^h 6.40 ±0.55 -2.07 ^g</td>
<td>8.14 ±0.45 10.0 ±0.53 1.90 ^ef 23.3 7.32 ±0.55 -0.82 -10.1</td>
</tr>
<tr>
<td>β-carotene (μg/mL)^1</td>
<td>0.153 ±02 0.168 ±0.06 0.015 0.249 ±0.10 0.096</td>
<td>0.229 ±0.02 1.04 ±0.06 0.812 ^e 355 1.44 ±0.10 1.21 ^ag</td>
</tr>
<tr>
<td>α-carotene (μg/mL)^1</td>
<td>0.099 ±02 0.089 ±0.01 -0.010 0.127 ±0.02 0.028</td>
<td>0.101 ±0.02 0.127 ±0.01 0.026 25.4 0.116 ±0.02 0.015 14.7</td>
</tr>
<tr>
<td>Lycopene (μg/mL)^1</td>
<td>0.148 ±02 0.163 ±0.02 0.015 0.210 ±0.03 0.062</td>
<td>0.153 ±0.02 0.256 ±0.02 0.103 ^ce 66.7 0.276 ±0.03 0.123 ^g 80.2</td>
</tr>
<tr>
<td>β-carotene (μg/mL)^1</td>
<td>0.763 ±05 0.696 ±0.05 -0.067 0.880 ±0.07 0.117</td>
<td>0.793 ±0.05 0.731 ±0.05 -0.062 -7.9 0.788 ±0.06 -0.005 -0.6</td>
</tr>
<tr>
<td>Retinol (μg/mL)^1</td>
<td>0.414 ±0.02 0.442 ±0.03 0.028 0.578 ±0.06 -0.164 ^h</td>
<td>0.449 ±0.03 0.480 ±0.03 0.031 6.9 0.522 ±0.06 0.073 ^h 16.2</td>
</tr>
<tr>
<td>Folate (ng/mL)^1</td>
<td>8.71 ±0.51 7.06 ±0.11 -1.65 7.68 ±0.13 -1.03</td>
<td>8.16 ±0.51 17.3 ±1.10 9.16 ^ae 112 22.4 ±1.26 14.2 ^ae 174</td>
</tr>
<tr>
<td>Homocysteine (nmol/L/mL)^2</td>
<td>9.91 ±0.50 9.18 ±0.43 -0.73 ^i 9.60 ±0.46 -0.31</td>
<td>10.2 ±0.48 8.33 ±0.41 -1.83 ^e -18.1 8.14 ±0.44 -2.02 de -19.9</td>
</tr>
<tr>
<td>Lipid Peroxides (μM)^1</td>
<td>0.376 ±02 0.286 ±0.02 -0.090 ^e 0.365 ±0.02 -0.011</td>
<td>0.370 ±0.02 0.270 ±0.02 -0.100 ^e -22.7 0.331 ±0.02 -0.039 ^h -10.5</td>
</tr>
<tr>
<td>8OHdG (ng/mL)^3</td>
<td>19.0 ±1.75 18.1 ±1.52 -0.90 ^h 14.1 ±1.58 -4.93 ^h</td>
<td>18.4 ±1.75 15.8 ±1.52 -2.55 -13.9 14.5 ±1.58 -3.88 ^h -21.1</td>
</tr>
</tbody>
</table>

^1 Measurements on serum samples. ^2 Measurements on plasma samples. ^3 Measurements on urine samples. ^a Different from placebo ^p<0.0001. ^b Different from placebo ^p<0.002. ^c Different from placebo ^p<0.0005. ^d Different from placebo ^p<0.03. ^e Different from baseline ^p<0.0001. ^f Different from baseline ^p<0.005. ^g Different from smokers ^p<0.005. ^h Different from smokers ^p<0.01. ^i Different from smokers ^p<0.02. ^j Different from smokers ^p<0.05.

### Table 3. Effects of active supplementation in smokers and non-smokers on various analytes from serum^1^, plasma^2^, and urine^3^ measured at baseline and 28 days

<table>
<thead>
<tr>
<th></th>
<th>Smokers n=17</th>
<th>Non-smokers n=13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 28 days Change %</td>
<td>Baseline 28 days Change %</td>
</tr>
<tr>
<td>α-tocopherol (μg/mL)^1</td>
<td>11.2 ±0.73 16.1 ±1.02 4.88 ^a 43.7</td>
<td>12.1 ±0.84 16.2 ±1.18 4.15 ^ae 34.4</td>
</tr>
<tr>
<td>β-carotene (μg/mL)^1</td>
<td>0.192 ±02 1.36 ±0.14 1.16 ^a 605</td>
<td>0.278 ^g ±0.03 1.55 ±0.16 1.27 ^g 456</td>
</tr>
<tr>
<td>Lycopene (μg/mL)^1</td>
<td>0.122 ±02 0.244 ±0.04 0.122 ^b 99.2</td>
<td>0.195 ^f ±0.02 0.320 ±0.05 0.125 ^c 64.1</td>
</tr>
<tr>
<td>Folate (ng/mL)^1</td>
<td>8.10 ±0.68 24.5 ±1.66 16.4 ^b 203</td>
<td>8.23 ±0.78 19.6 ±1.89 11.4 ^b 138</td>
</tr>
<tr>
<td>Homocysteine (nmol/L/mL)^2</td>
<td>10.6 ±0.65 8.32 ±0.58 -2.28 ^a -21.5</td>
<td>9.59 ±0.74 7.91 ±0.67 -1.68 ^b -17.5</td>
</tr>
<tr>
<td>Lipid Peroxides (μM)^1</td>
<td>0.385 ±0.02 0.341 ±0.02 -0.044 ^d -11.4</td>
<td>0.351 ±0.02 0.319 ±0.03 -0.032 -9.1</td>
</tr>
<tr>
<td>8OHdG (ng/mL)^3</td>
<td>20.9 ±2.20 17.5 ±2.03 -3.42 ^d -16.3</td>
<td>15.0 ±2.63 10.5 ±2.32 -4.47 ^h -29.9</td>
</tr>
</tbody>
</table>

^a Different from baseline ^p<0.0001. ^b Different from baseline ^p<0.005. ^c Different from smokers ^p<0.01. ^d Different from smokers ^p<0.02. ^e Different from smokers ^p<0.05.
Serum folate levels increased 112% and 174% after supplementation for 14 and 28 days respectively compared to baseline (p<0.0001; Figure 1). Reflecting the rise in serum folate, plasma homocysteine decreased 18.1% after 14 days supplementation (p<0.0001) and continued to decrease at 28 days (p<0.0001; Fig 2). Plasma homocysteine concentrations were correlated negatively with serum folate levels (r=0.268, p=0.0004). Figure 3 depicts changes in plasma homocysteine for each subject, showing that 27 out of 30 subjects (90%) experienced a decrease in homocysteine, with 20 out of 30 subjects exhibiting a 15% or greater decrease in homocysteine levels after 28 days of active supplementation.

Serum lipid peroxides decreased significantly compared to baseline levels after active supplementation for 14 days (p<0.0001) and 28 days (p<0.05), but were not significantly different than placebo (Table 2). Urine concentrations of 8-OHdG were also decreased after 28 days in the active group (p<0.05), but a similar decrease occurred in the placebo group. The 8-OHdG generating capacity of urine in the active group also showed a decreasing trend at 28 days (p<0.07; data not shown).

The effects of active supplementation on smokers (n=17) compared to non-smokers (n=13) was also examined (Table 3). Smokers exhibited significantly lower baseline levels of β-carotene (p<0.02) and lycopene (p<0.01) than non-smokers. After 28 days, while both smokers and non-smokers showed significant increases from baseline in serum α-tocopherol, β-carotene, lycopene and folate (Table 3), smokers exhibited greater increases but only the comparative change in α-tocopherol reached significance (p<0.005). Likewise, homocysteine levels decreased significantly from baseline in both smokers (p<0.0001) and non-smokers (p<0.005) while the decrease in lipid peroxides was only significant in smokers (p<0.05). Urine levels of 8OHdG were significantly different between smokers and non-smokers after 28 days (p<0.05) but decreases from baseline did not reach significance in either group.

**DISCUSSION**

This study showed that administration of a mixed fruit and vegetable concentrate produced from dehydrated juice provided a valuable source of bioavailable antioxidants and other micronutrients leading to significant increases in blood concentrations of protective antioxidants and folate, and a reduction in serum homocysteine levels. Supplementation with fruit and vegetable concentrates over a 28-day period produced significant increases in serum antioxidants such as α-tocopherol, β-carotene and lycopene. As has been shown previously, enhanced
antioxidant status can help to reduce damaging oxidative processes.\textsuperscript{42,43,48} In addition, subjects in the active group experienced significant lowering of plasma homocysteine compared to the placebo group at the end of this 28 day investigation. Serum levels of \(\beta\)-carotene rose significantly in the present study in both smokers and non-smokers (vs. placebo; \(p<0.0001\)). Increases of a similar magnitude have been observed in previous studies in the USA,\textsuperscript{53} in Australia\textsuperscript{48} and in Austria,\textsuperscript{49} demonstrating consistently superior bioavailability of \(\beta\)-carotene from fruit and vegetable concentrates. This is in contrast to large variations in reported bioavailability of \(\beta\)-carotene from dietary sources.\textsuperscript{50-52}

Many factors may contribute to the variable uptake of \(\beta\)-carotene and other carotenoids, but is thought to be mainly due to the sequestering within cellular matrices such as chloroplasts.\textsuperscript{50} Consistent with this explanation are studies contrasting the bioavailability of \(\beta\)-carotene from fresh tomatoes compared to heat-processed tomatoes in the form of tomato paste.\textsuperscript{53} As the plant cell walls are disrupted during the juicing process, the bioavailability of \(\beta\)-carotene shown in this study can likewise be explained by the methods used to manufacture the fruit and vegetable juice concentrates. In addition, there may be additive and/or synergistic effects of the phytochemicals in fruits and vegetables responsible for their antioxidant activity,\textsuperscript{54} so a supplement derived from these sources could mimic this effect.

Serum levels of lycopene also showed significant increases in the active group vs. baseline \(p<0.0005\). Others have observed increased dietary levels of lycopene associated with a 45% reduction in prostate cancer,\textsuperscript{55} reduction in most forms of digestive cancer, including cancer of the mouth, esophagus, stomach, intestine, colon and rectum.\textsuperscript{56} In addition, increased serum levels of \(\beta\)-carotene or lycopene have been reported to reduce the rate of cancer at all sites or the rate of colorectal cancer in Japanese populations.\textsuperscript{8}

Levels of \(\alpha\)-tocopherol increased significantly in the fruit and vegetable juice concentrate group vs. placebo; \(p<0.0001\). High intakes of \(\alpha\)-tocopherol have been linked to reductions in coronary risk,\textsuperscript{57,59} but this antioxidant may have other benefits such as improved cellular immunity, as observed in elderly and in cancer patients.\textsuperscript{50,61} Moreover, elderly subjects supplemented with fruit and vegetable juice concentrates for 80 days experienced significant increases in serum \(\alpha\)-tocopherol (from 22.56 to 28.75 \(\mu g/mL\); \(p=0.003\) and also reported improvement in several markers of immune function, including increased natural killer cell activity and improved IL-2 levels, regardless of smoking status.\textsuperscript{44}

Folate has emerged as a very important vitamin with multiple functional effects, since it is an essential nutrient for cell development.\textsuperscript{62} In particular, methyltetrahydrofolate participates in methylation reactions such as the synthesis of methionine from homocysteine.\textsuperscript{62} Elevated homocysteine levels are also thought to be a risk factor for CVD.\textsuperscript{63} In this study, serum folate levels rose significantly (\(p<0.0001\)) in the group given fruit and vegetable juice concentrates compared with the placebo group. There was a corresponding significant reduction in plasma homocysteine levels in the active compared to the placebo group (\(p<0.0001\)). As expected, these changes in plasma homocysteine were negatively correlated with the increase in serum folate concentrations (\(r=0.268; p=0.0004\)). Significant increases in folate and decreases in homocysteine levels have been observed in other studies in which fruit and vegetable juice concentrates were supplemented.\textsuperscript{56,64} Furthermore, similar associations have been found in other studies in which fruit and vegetable dietary intake was increased.\textsuperscript{55,66}

The results for 8-OHdG levels are not significantly lower in the group receiving the supplement than the placebo group, though reductions of 14 and 21% compared to baseline were recorded after 14 and 28 days of supplementation, respectively. It is tempting to speculate that had this trend continued, then a significant result may have been obtained in a trial of longer duration. 8-OHdG levels are indicative of oxidative modification of cellular DNA, and thus lower levels of 8-OHdG following supplementation with fruit and vegetable concentrates substantiate the potential protective effects of serum antioxidants on DNA integrity. A previous study employing mixed fruit and vegetable juice concentrates in elderly volunteers showed a four-fold decrease in damage to peripheral lymphocyte DNA from baseline levels following supplementation for 80 days (\(p<0.0001\)) – an effect that was observed in both smokers and non-smokers.\textsuperscript{44} In their study, the more sensitive comet assay was used as a measure of DNA chain breakage compared to the appearance of 8-OHdG in urine, which requires significant DNA degradation.

It is interesting to note that serum vitamin C levels rose modestly, but significantly, after 14 days in the active group (\(p<0.0005\) in this study, but fell to just below baseline levels by day 28 of the trial. There are many potential explanations for this result, including variations in dietary and/or smoking patterns throughout the trial, degradation of vitamin C in serum samples, or just statistical variability among the data samples. However, in a previous placebo-controlled study using fruit and vegetable juice concentrates, vitamin C levels increased significantly vs. placebo treatment (\(p=0.002\)).\textsuperscript{48} Another potential explanation is that baseline vitamin C levels were moderately high at baseline (8.14 and 8.47 \(\mu g/mL\)) in the study reported here, which is close to plateau levels at which the threshold for urinary excretion comes into play.\textsuperscript{67}

Lipid peroxidation in serum has been used as a measure of oxidative stress.\textsuperscript{68,69} Changes in lipid peroxides have been negatively correlated with increases in serum antioxidants.\textsuperscript{42} In this study, lipid peroxides were significantly decreased in the active group compared to baseline levels at 14 days (\(p<0.0001\)) and at 28 days (\(p<0.05\), but did not reach significance compared to the placebo group. This can be attributed to the large variability between individuals, but the trend is consistent with previous studies measuring the effects of supplementation with fruit and vegetable juice concentrate capsules on reduction in lipid peroxides,\textsuperscript{42} reduction in malondialdehyde\textsuperscript{63} and increases in ferric-reducing ability of plasma (FRAP) concentrations.\textsuperscript{48}

Cell structures and macromolecules are susceptible to damage from free radicals generated by normal metabolic
processes, but are protected by antioxidant defense systems. This defense system declines with age and is compromised by forms of oxidative stress including exposure to tobacco smoke. This is of particular concern in populations with a high proportion of smokers, such as in Japan, where about 51% of men smoke and the prevalence of smoking among women, once considered almost taboo, has risen dramatically in the last decade to nearly 15%.70

Active smokers have greater than 25% lower serum concentrations of ascorbic acid, α-carotene, β-carotene, and cryptoxanthin compared to non-smokers.37,38 The associations with active smoking also appear to be true for passive smoking.71 In this study, smokers showed lower levels of folic acid, α-tocopherol, β-carotene (p<0.02), and lycopene (p<0.01) with the latter two reaching significance. Compromised antioxidant status in smokers has been correlated with elevated serum 8OHdG,39 lipid peroxides,72 malondialdehyde70 and impaired endothelial function.73,74 Both lipid peroxides and urine 8OHdG were higher in smokers than non-smokers in this study and supplementation reduced levels of both markers but the differences and changes did not reach significance. Compared with non-smokers, serum folate levels in smokers is reduced and has been correlated with increased homocysteine levels; however, decreased folate levels in smokers did not correlate with dietary intake of folate.75 In this study folate levels in smokers increased significantly (p<0.0001) while homocysteine decreased (p<0.0001).

Lower serum antioxidant and folate levels in smokers appears to be attributable to two distinct and independent factors. First, oxidative stress from cigarette smoke has been reported to require increased utilization and subsequent depletion of α-tocopherol,38,76 vitamin C,72 carotenoids,37,38,77 and folate.75 This increased turnover and degradation of antioxidants in smokers is reflected in various measures of DNA and cellular damage previously referenced. Second, smokers have been reported to have lower dietary intakes of antioxidants and folate.10,11 In particular, lower consumption of fruits and vegetables, a major source of antioxidants, appears to also contribute to the poor antioxidant status of smokers. Since fruits and vegetables are a good source of β-carotene, vitamin C and vitamin E, in particular, these antioxidants have been highlighted as having a protective role for the prevention of cancer and CVD.78 For example, when disease risk in populations was correlated with plasma levels of nutrients, the quintiles with the highest level of β-carotene (>0.22 µg/mL), α-tocopherol (>12.9 µg/mL) and vitamin C (>8.8 µg/mL) had the lowest risk for cancer and CVD.79,81 In the present study, after only 14 days of supplementation with fruit and vegetable juice concentrates, levels of serum β-carotene (1.04 µg/mL), α-tocopherol (14.62 µg/mL) and Vitamin C (10.04 µg/mL) increased to levels which exceed the above cited concentrations correlated with minimum risk of disease, with no difference between smokers and non-smokers.

Although dietary intake of fruits and vegetables appears to correlate with serum levels of the well-known antioxidants (β-carotene, vitamin C and vitamin E), other antioxidants such as carotenoids, phenolic compounds, sulfides, flavonoids and lignans may play a prominent role in modulating oxidative stress.23-25 These compounds are thought to contribute to the high antioxidant capacity observed in certain fruits and vegetables, with some flavonoids having many times greater antioxidant activity than vitamins C or E.82,83 This has been demonstrated by comparing the antioxidant capacity of serum using three different methods in elderly women consuming different foods, drinks or vitamin C supplements.83 Meals including strawberries, spinach or red wine increased serum antioxidant capacity to an extent equivalent or greater than a large (1250 mg) dose of vitamin C. Furthermore, the total antioxidant capacity of a medium-sized apple was found to be equivalent to 2250 mg of vitamin C, even though the vitamin C content of the apple was less than 10 mg.84 This activity, obtained from whole apple extracts, was attributed to phenolic acids and flavonoids. These studies suggest that the protective effects of fruits and vegetables result from a synergistic effect of the numerous different antioxidants present, which may work in an integrated antioxidant defense system.85,86 Further demonstration of a functional antioxidant network has been shown in a recent study which correlated total dietary antioxidant intake to plasma levels of carotenoids, thiols and tocopherols.87 Although dietary carotenoids were only a minor component of the total antioxidants consumed, plasma levels were significantly correlated with total antioxidant intake. These results indicate that many antioxidants function in an integrated network, and higher than expected carotenoid levels result from sparing, salvaging or recycling of these molecules by other dietary antioxidants.87

In the present study, the results are also suggestive of synergistic interactions among the bioactive phynonutrients present in fruit and vegetable juice concentrates compared to the placebo. Although modest levels of the key antioxidants were provided in the active group, the significant serum increases observed may be reflective of an interactive network which spares or regenerates the marker antioxidants. CONCLUSIONS

Fruits and vegetables are rich sources of antioxidant nutrients and folate, which play a critical role in maintaining health and reducing the likelihood of diseases such as CVD and cancers. However, some individuals still find eating significant quantities of fruits and vegetables difficult to achieve. Supplements derived from dehydrated fruit and vegetable juices have proved to be effective in raising the plasma levels of most of the nutrients studied and may be beneficial in improving the nutritional status in those individuals who do not eat sufficient fruits and vegetables, offering synergistic benefits from the multiple components. It is noteworthy that active smokers with increased levels of oxidative stress, after a short period of supplementation, showed improvements in antioxidant status comparable to those seen in non-smokers.

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Effects of fruit and vegetable juice concentrates on antioxidants folate and homocysteine

Four week supplementation with mixed fruit and vegetable juice concentrates increased protective serum antioxidants and folate and decreased plasma homocysteine in Japanese subjects

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補充四週濃縮綜合蔬果汁，可增加日本受試者血清中保護性血清抗氧化物及葉酸，降低其血漿同半胱胺酸

攝取水果及蔬菜與癌症及心血管疾病等慢性疾病的危險性具有負相關，其益處可歸因於各種保護性抗氧化劑、胡蘿蔔素及植物營養素。本研究目的為評估補充脫水濃縮綜合蔬果汁的(Juice Plus+®)對血清抗氧化劑及葉酸狀況、血漿同半胱胺酸濃度及氧化壓力標記及 DNA 傷害之影響。招募日本受試者（n=60；年齡 27.8 歲；BMI 22.1）參與本雙盲安慰劑控制研究，並隨機分成兩組各 30 名，配對性別、年齡、BMI 及抽菸狀況（39 名男性，22 名抽菸者；21 名女性，13 名抽菸者）。給予受試者含有綜合蔬果汁濃縮物或是配對安慰劑的膠囊 28 天。在研究開始、第 14 天及第 28 均收集血液及尿液樣本進行分析測試。與安慰劑組比較，28 天補充顯著升高血清 beta-胡蘿蔔素 528% (p<0.0001)、蕃茄紅素 80.2% (p<0.0005)及 alpha 生育醇 39.5% (p<0.0001)。血清葉酸增加 174.3% (p<0.0001)，而與其相關的血漿同半胱胺酸降低 19.9% (p<0.03)。與研究開始相比，氧化壓力的指標血清脂質過氧化物降低-10.5% (p<0.02)及尿中 8OHdG 減少 21.1% (p<0.02)。評估抽菸者(n=17)，28 天補充的結果顯示有類似的變化。結論：在不改變飲食的情況下，蔬果汁濃縮膠囊補充劑證明為植物營養素的高生物活性來源。重要的抗氧化劑提高至理想的濃度，因氧化壓力標記下降，並使疾病危險性降低；葉酸狀況的改善伴隨著同半胱胺酸的降低。與非抽菸者比較，這些益處在抽菸者身上顯示類似的結果。

關鍵字：水果、蔬菜、抗氧化物、葉酸、同半胱胺酸、胡蘿蔔素、抽菸者。