Effect of *Citrus Aurantium* Combined with Caffeine and/or Tea Catechins on Body Fat Accumulation and Its Safety in Rats

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**Summary** We investigated the weight loss efficacy and safety of *Citrus aurantium* (CA, 1,000 mg/kg diet) along with usual levels of adrenergic stimulants in foods, *i.e.* caffeine (100 mg/kg diet) and/or tea catechins (500 mg/kg diet), in rats for 44 or 45 days. Even in combination with caffeine and tea catechins, the suppressive effect of CA against body fat accumulation was negligible, whereas no deleterious influences concerning cardiotoxicity were observed. Thus, although the efficacy of CA for weight loss seems to be questionable, no safety problems may occur even in people who habitually consume coffee and tea. However, it is noteworthy that the intake of CA markedly elevated the urinary excretion of adrenaline, and this was not affected by intake of caffeine and/or tea catechins at the usual levels. Therefore, the safety of CA intake with high levels of caffeine and tea catechins, especially in people at risk of heart disease, remains to be elucidated further.

**Key Words**: *Citrus aurantium*, caffeine, tea catechins, body fat accumulation, safety

**Introduction**

Herbal supplements containing *Citrus aurantium* (CA) extracts have recently been marketed for claiming weight loss (reviewed in references [1–3]). Similarly to ephedra, CA contains several adrenergic amines, and the most abundant one is synephrine [4]. Synephrine is structurally similar to adrenaline and noradrenaline (Fig. 1), and has been suggested to stimulate thermogenesis and lipolysis in adipocytes through the activation of β-adrenergic receptor and thereby facilitate weight loss. Some clinical trials showed that CA-containing combination products lost body weight and/or body fat [5–7], but these results are likely to arise from ingredients other than CA, particularly ephedrine or caffeine. Therefore, little

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**Fig. 1.** Chemical structures of synephrine, adrenaline and noradrenaline.
evidence supports the efficacy of CA itself for weight loss in human at present.

On the other hand, synephrine acts as an α- and β-
adrenergic antagonist [8, 9] and thus is predicted to have hemodynamic effects, with potential implication risks for adverse effects. In fact, there were one case report of acute lateral-wall myocardial infarction in a 55-year-old woman [10], one case report of exercise-induced syncope with QT prolongation in a 22-year-old woman [11], and one case report of ischemic stroke in a 38-year-old man [12], which were associated with use of ephedra-free dietary supplements containing CA. In addition, Calapai et al. [13] reported that in rats the oral administration of CA alone significantly reduced body weight gain but high dose of CA caused ventricular arrhythmias and death.

Therefore, we have previously investigated the safety of CA alone and its suppressive effect against body fat accumulation in rats [14]. Dietary CA intakes less than or equal to 1,000 mg CA/kg diet (recommended daily intake of CA products is nearly equivalent to 200 mg CA/kg diet) did not suppress body weight gain or body fat accumulation, and not cause any biochemical and histopathological changes concerning toxicity. Thus, CA alone seemed to be ineffective against body fat accumulation but safe. However, an excessive intake of 5,000 mg/kg diet of CA suppressed significantly perirenal fat accumulation, but induced concomitantly heart weight loss and increased plasma levels of adrenaline and dopamine and urinary excretion of adrenaline [14].

There are some food components that could stimulate adrenergic antagonists, e.g. caffeine and catechin-polyphenols [15]. These stimulants may be synergistic with CA for weight loss, but may also increase the risk of cardiotoxicity as Marcus and Grollman [16] and Jordan et al. [17] have warned. It needs to be noted that some weight-loss products contain CA together with such stimulants at the same time. In 2004, Health Canada banned a certain product containing synephrine, caffeine, catechins and other stimulants [18]. Therefore, in the present study, we investigated the influences of simultaneous intake of CA with caffeine and/or tea catechins on body fat accumulation and safety in rats.

Materials and Methods

Animals, diets and feeding trial

The experiments were done in accordance with the guidelines of the Animal Committee of the Incorporated Administrative Agency, National Institute of Health and Nutrition (Tokyo, Japan).

Male Sprague-Dawley rats (CLEA Japan, Tokyo, Japan), 8 week of age and weighing 260–290 g, were housed individually at a controlled temperature of 22 ± 1°C and humidity of 50–60% with a 12-h light:dark cycle. They were first fed the AIN-93G purified diet for laboratory rodents [19] and had access to water ad libitum for 7 days. The rats were then randomly assigned by weight and weight gain to 7 diet groups, (i) control group, (ii) CA group, (iii) caffeine (Caf) group, (iv) CA + Caf group, (v) tea catechins (Cat) group, (vi) CA + Cat group and (vii) CA + Caf + Cat group, and raised for 44 or 45 days. Food and water were available ad libitum.

The composition of the high-fat experimental diets, based on the AIN-93G purified diet for laboratory rodents, is shown in Table 1. Lipid content of the diets was 20 w/w % and 37.5% of total energy. Powder extract of CA was purchased from Exquim, S.A. (Barcelona, Spain); this product contains 6.4% of synephrine according to manufacturer’s data sheet and we confirmed this by HPLC [14]. Caffeine anhydride was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Polyphenon-60 (green tea catechins, Lot. 0409171) was purchased from Mitsui Norin Co., Ltd. (Tokyo, Japan); this product contains 60.3% of catechins (27.2% of (−)-epigallocatechin gallate, 15.2% of (−)-epigallocatechin, 7.7% of (−)-epicatechin gallate, 6.8% of (−)-epicatechin, 2.9% of (−)-gallocatechin gallate and 0.5% of (−)-catechin gallate) according to manufacturer’s data sheet and 7.4% of caffeine measured by HPLC as reported by Goto et al. [20].

After 13, 28 and 39 days, 24-hr urine was collected using metabolic cages and was stored at 4°C until use. At the end of the experiment (44 or 45 days), the rats were killed by cardiac puncture. Their liver, kidney, heart, testis, spleen, lung, and perirenal and epididymal fat pads were promptly excised, washed with isotonic saline and weighed. The hearts of all rats were fixed with 10% formalin neutral buffer solution, pH 7.4, and histopathological examinations were performed after hematoxylin-eosin staining. Serum and plasma were separated by centrifugation at 2,700 × g at 4°C for 15 min and stored at −80°C until use.

Assay procedures

Serum inhibin-B concentration was determined by a sandwich EIA kit (Oxford Bio-Innovation Ltd., Oxfordshire, UK). Other serum parameters determined were as follows: total protein, albumin, ratio of albumin/globulin (A/G), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine, blood urea nitrogen (BUN), glucose, glycosylated albumin, triacylglycerol (TG), phospholipids (PLs), free fatty acids, total cholesterol, HDL-cholesterol, total bilirubin, triiodothyronine (T3) and thyroxine (T4); plasma parameters were as follows: adrenaline, noradrenalin and dopamine; urinary parameters were as follows: adrenaline, noradrenaline, dopamine and homovanillic acid. These biochemical parameters were measured with commercially available kits.
Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer post-hoc test for multiple comparisons. The p-values less than 0.05 were considered statistically significant. Data that failed the Levene’s test for equality of variances were log-transformed to stabilize variances before analysis.

Results

The rats consumed 22.8–26.7 g food/day and gained 4.8–5.7 g BW/day over the 44- or 45-day-experiment (Table 2). There were no significant differences in the food intake and body weight gain among the groups. The relative weights of liver, kidney, testis, spleen, lung, and perirenal and epididymal fat pad did not change significantly in any of the treatment groups.

The hearts showed no histopathological abnormalities attributable to three dietary components of CA, caffeine and tea catechins, even in the CA + Caf + Cat group (data not shown). The plasma concentrations of adrenaline, noradrenaline and dopamine were not significantly different between the groups (Table 3). Furthermore, no obvious changes in the serum biochemical parameters were observed in any of the treatment groups (Table 4). The variations of serum albumin concentration were within the normal range. The 24-h urinary excretion of adrenaline was already increased in the CA intake groups on the 13th day of the experiment, and remained elevated thereafter (Table 5). Caffeine and tea catechins did not further increase the urinary excretion of adrenaline even in the combination with CA. On the other hand, there were not notable changes in the urinary excretion of noradrenaline, dopamine and homovanillic acid between the groups.

Discussion

The dose of CA used in this study was 1,000 mg/kg diet, which was no observed adverse effect level (NOAEL) of CA alone in our previous study [14]. CA intakes calculated using final body weights in the present study were ca. 40–50 mg CA/kg BW/day (ca. 3 mg synephrine/kg BW/day); this is ca. 2,000–2,500 mg CA/day (ca. 150 mg synephrine/day) for a 50 kg BW human, and 2–25 times excess of recommended daily intake on the labels of many CA products (100–1,000 mg CA/day). In a similar manner, caffeine intakes were ca. 2–6 mg caffeine/kg BW/day; this is ca. 100–300 mg caffeine/day for a 50 kg BW human, and...
In combination with usual levels of caffeine and tea, synephrine intake was equivalent to caffeine level in a few cups of coffee. Tea catechins intakes were ca. 14 mg tea catechins/kg BW/day; this is ca. 700 mg tea catechins/day for a 50 kg BW human, and equivalent to tea catechins level in ca. 1 liter of green tea. In our present study, caffeine or tea catechins alone did not suppress body fat accumulation although they have been reported to reduce body fat mass. This may be because the doses used in this study were below an effective dose, e.g., 250 mg caffeine/kg diet, in rats, reported by Kobayashi-Hattori et al. [21], and 2,000 mg tea catechins/kg diet, in mice, reported by Murase et al. [22], respectively.

Our results suggest that excess level of CA did not suppress body weight gain and body fat accumulation even in combination with usual levels of caffeine and tea catechins in rats. However, Colker et al. [5] reported that human subjects (BMI>25 kg/m²) received CA (975 mg/day), caffeine (528 mg/day) and St. John’s wort (900 mg/day) for 6 weeks with exercise and mild caloric restriction lost body weight and body fat significantly. In addition, Gougeon et al. [23] reported that human subjects received single dose of CA capsules (containing 26 mg synephrine and 10.5 mg other adrenergic amines) were significantly higher in energy expenditure compared with the baseline. Carpéné et al. [24] showed that adrenergic agonists stimulated lipolysis in white fat cells through the activation of β1-adrenergic receptor in rats, but through the activation of β1- and/or β2-adrenergic receptor in humans, while synephrine activated β1- and/or β2-adrenergic receptor rather...
than β1-adrenergic receptor in rats. Thus, the suppressive effect of synephrine against body fat accumulation in rats may not directly apply to that in humans, and vice versa. However, there is still little evidence that CA itself would be effective for weight loss in humans, although many products for weight loss containing CA have been widely marketed. In addition, Jordan et al. [9] reported that synephrine was more than 4 orders of magnitude less active β1- and β2-antagonist compared to noradrenaline. Therefore, CA seems not to be effective for weight loss and body fat accumulation in humans.

On the other hand, the intake of CA even in combination with caffeine and tea catechins did not induce cardiotoxicity. In addition, serum and plasma biochemical parameters including plasma adrenaline and dopamine, which were increased by the large excess intake of CA in our previous study [14], did not change in any of the treatment groups. We also measured inhibin-B as a marker of spermatogenesis, which we found to be decreased in male rats by an excess intake of CA combined with caffeine and tea catechins. Prolonged elevation of catecholamines in the circulation begins to initiate deleterious effects, particularly on the heart [27]. Elevated circulating adrenaline due to the intake of CA seems to be excreted promptly into urine, and usual levels of caffeine and tea catechins did not affect the circulating level of adrenaline and its excretion, and thereby no cardiotoxicity may occur under the current experimental conditions.

Table 4. Influence of dietary *Citrus aurantium* (CA) combined with caffeine (Caf) and/or tea catechins (Cat) on serum biochemical indicators in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>CA</th>
<th>Caf</th>
<th>CA + Caf</th>
<th>Cat</th>
<th>CA + Cat</th>
<th>CA + Caf + Cat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/liter)</td>
<td>60.8 ± 2.2NS</td>
<td>58.5 ± 1.5</td>
<td>60.8 ± 1.9</td>
<td>60.6 ± 2.1</td>
<td>59.3 ± 1.5</td>
<td>59.8 ± 0.4</td>
<td>59.3 ± 1.7</td>
</tr>
<tr>
<td>Albumin (g/liter)</td>
<td>29.5 ± 1.0b</td>
<td>28.8 ± 0.8a</td>
<td>30.5 ± 0.5b</td>
<td>30.3 ± 1.3b</td>
<td>29.3 ± 0.5b</td>
<td>29.8 ± 0.8b</td>
<td>28.6 ± 0.5a</td>
</tr>
<tr>
<td>Ratio of albumin/globulin</td>
<td>0.95 ± 0.05NS</td>
<td>0.97 ± 0.05</td>
<td>1.00 ± 0.06</td>
<td>1.01 ± 0.04</td>
<td>0.97 ± 0.05</td>
<td>0.98 ± 0.08</td>
<td>0.94 ± 0.05</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/liter)</td>
<td>87.7 ± 11.9NS</td>
<td>86.3 ± 11.6</td>
<td>85.3 ± 7.9</td>
<td>79.1 ± 11.8</td>
<td>91.7 ± 12.4</td>
<td>91.7 ± 7.7</td>
<td>86.3 ± 12.8</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/liter)</td>
<td>28.3 ± 4.6NS</td>
<td>30.3 ± 8.3</td>
<td>24.8 ± 5.5</td>
<td>26.4 ± 6.1</td>
<td>28.3 ± 6.0</td>
<td>25.5 ± 1.9</td>
<td>23.4 ± 2.3</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/liter)</td>
<td>405 ± 40NS</td>
<td>367 ± 66</td>
<td>361 ± 53</td>
<td>417 ± 114</td>
<td>349 ± 60</td>
<td>461 ± 79</td>
<td>418 ± 68</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/liter)</td>
<td>655 ± 275NS</td>
<td>747 ± 290</td>
<td>705 ± 215</td>
<td>594 ± 155</td>
<td>972 ± 351</td>
<td>608 ± 131</td>
<td>798 ± 367</td>
</tr>
<tr>
<td>Creatinine (µmol/liter)</td>
<td>29.3 ± 1.5NS</td>
<td>30.1 ± 3.7</td>
<td>26.1 ± 1.5</td>
<td>26.1 ± 3.3</td>
<td>27.8 ± 0.9</td>
<td>27.0 ± 4.9</td>
<td>28.3 ± 1.0</td>
</tr>
<tr>
<td>Urea nitrogen (mmol/liter)</td>
<td>4.9 ± 0.9NS</td>
<td>4.7 ± 0.5</td>
<td>4.6 ± 0.6</td>
<td>4.4 ± 0.7</td>
<td>4.9 ± 0.4</td>
<td>4.5 ± 0.4</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>Glucose (mmol/liter)</td>
<td>13.7 ± 1.4NS</td>
<td>13.5 ± 1.7</td>
<td>13.1 ± 1.4</td>
<td>12.7 ± 1.8</td>
<td>12.6 ± 0.4</td>
<td>13.5 ± 1.5</td>
<td>13.0 ± 1.3</td>
</tr>
<tr>
<td>Glycosylated albumin (%)</td>
<td>8.47 ± 0.48NS</td>
<td>7.45 ± 1.28</td>
<td>6.22 ± 1.96</td>
<td>6.54 ± 1.57</td>
<td>7.55 ± 1.02</td>
<td>6.72 ± 1.73</td>
<td>7.34 ± 1.17</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/liter)</td>
<td>1.79 ± 0.57NS</td>
<td>1.51 ± 0.50</td>
<td>1.53 ± 0.59</td>
<td>1.74 ± 1.07</td>
<td>1.48 ± 0.61</td>
<td>1.44 ± 0.73</td>
<td>1.62 ± 1.24</td>
</tr>
<tr>
<td>Phospholipids (mmol/liter)</td>
<td>1.98 ± 0.28NS</td>
<td>1.61 ± 0.25</td>
<td>1.93 ± 0.37</td>
<td>1.97 ± 0.49</td>
<td>1.64 ± 0.38</td>
<td>1.69 ± 0.36</td>
<td>1.69 ± 0.40</td>
</tr>
<tr>
<td>Free fatty acids (mmol/liter)</td>
<td>0.72 ± 0.16NS</td>
<td>0.64 ± 0.16</td>
<td>0.66 ± 0.13</td>
<td>0.64 ± 0.20</td>
<td>0.69 ± 0.20</td>
<td>0.60 ± 0.21</td>
<td>0.58 ± 0.14</td>
</tr>
<tr>
<td>Total cholesterol (mmol/liter)</td>
<td>1.85 ± 0.28NS</td>
<td>1.48 ± 0.22</td>
<td>1.80 ± 0.45</td>
<td>1.91 ± 0.33</td>
<td>1.44 ± 0.29</td>
<td>1.53 ± 0.18</td>
<td>1.61 ± 0.33</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/liter)</td>
<td>0.72 ± 0.07NS</td>
<td>0.63 ± 0.08</td>
<td>0.70 ± 0.10</td>
<td>0.71 ± 0.12</td>
<td>0.61 ± 0.11</td>
<td>0.65 ± 0.10</td>
<td>0.62 ± 0.07</td>
</tr>
<tr>
<td>Total bilirubin (µmol/liter)</td>
<td>2.0 ± 0.7NS</td>
<td>1.7 ± 0.0</td>
<td>2.9 ± 0.9</td>
<td>1.7 ± 0.0</td>
<td>2.3 ± 0.9</td>
<td>2.3 ± 0.9</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>Triiodothyronine (nmol/liter)</td>
<td>1.08 ± 0.12NS</td>
<td>1.01 ± 0.16</td>
<td>1.03 ± 0.17</td>
<td>1.06 ± 0.17</td>
<td>1.07 ± 0.11</td>
<td>1.15 ± 0.16</td>
<td>1.01 ± 0.14</td>
</tr>
<tr>
<td>Thyroxine (nmol/liter)</td>
<td>59.0 ± 3.6NS</td>
<td>50.4 ± 9.3</td>
<td>57.7 ± 7.3</td>
<td>57.7 ± 9.4</td>
<td>54.5 ± 3.4</td>
<td>57.3 ± 5.1</td>
<td>53.7 ± 9.8</td>
</tr>
<tr>
<td>Inhibin-B (ng/liter)</td>
<td>32.8 ± 11.2NS</td>
<td>35.7 ± 9.8</td>
<td>30.3 ± 10.9</td>
<td>33.2 ± 10.3</td>
<td>41.2 ± 11.8</td>
<td>34.4 ± 10.2</td>
<td>34.2 ± 12.3</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of 6–7 rats in each group. Means in a row that are not followed by a common superscript letter are different, p<0.05. NS, not significant.
conditions. Therefore, if the instructions on the labels of CA products are properly followed, no safety problems may occur even in combination with habitual intakes of caffeine and tea catechins.

Haller et al. [28] have reported that single dose of Xenadrine EFX, a product containing 5.5 mg synephrine in combination with other stimulants such as green tea extract, yerba mate, tyramine, etc., induced more intense cardiovascular stimuli compared with Advantra Z, a product containing 46.9 mg synephrine alone, in a clinical trial; stimulants included in Xenadrine EFX may increase cardiovascular effect of synephrine. Furthermore, Dulloo et al. [29] showed that a green tea extract containing 50 mg caffeine and 90 mg epigallocatechin gallate increased the 24-h energy expenditure and 24-h urinary noradrenaline excretion in humans; caffeine and tea catechins seem to affect the sympathetic nervous system. In addition, there are some case reports of adverse effects associated with use of ephedra-free dietary supplements containing CA (see Introduction). Therefore, the safety of CA with higher levels of caffeine and tea catechins needs to be examined further.

The characteristic observations in this and our previous studies are the elevated urinary excretion of adrenaline, but not noradrenaline, in rats fed CA. In addition, an intake of a CA product also increased excretion of adrenaline in humans [23]. As mentioned above, elevated adrenaline exceeding physiological concentrations may cause cardiotoxicity, and adrenochrome, an oxidative metabolite of adrenaline, is postulated to be the biochemical initiators of cardiotoxicity, rather than adrenaline per se [27]. It is noteworthy that synephrine was reported to be oxidized to adrenochrome by mushroom tyrosinase, via adrenaline as an intermediate [30]. If the increased adrenaline is a metabolite of synephrine catalyzed by mammalian tyrosinase, adrenochrome may be formed at the same time. Hence, the long-term safety evaluation of CA is left to be investigated further.

The results obtained here show that the intake of CA combined with a usual level of caffeine and tea catechins failed to suppress body fat accumulation, whereas no deleterious effect was observed in this short-term study in rats. Therefore, as far as recommended daily intakes designated on the labels of CA products are followed, no safety problems may occur even in people who habitually consume coffee and tea. However, the safety evaluation of CA intake along with high levels of caffeine and tea catechins, especially in people at risk for heart disease, needs to be elucidated further.

Acknowledgments

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