Effects of Dietary Apple Polyphenol on Adipose Tissues Weights in Wistar Rats

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Abstract: In this study, we investigated whether dietary apple polyphenol (APP) had an effect on adipose weights. Twenty-four Wistar male rats (10 weeks of age) were assigned to three groups: (1) the 5%APP group (diet containing 5% APP, N=8); (2) the 0.5%APP group (diet containing 0.5% APP, N=8); and (3) the control group (N=8) so that average weights of the groups were the same. After a three-week experimental period, adipose tissue weights were measured. Pathological and plasma characteristics were also examined. Retroperitoneal and epididymal adipose tissue weights in the 5%APP group were significantly lower than those of the control (P<0.05). Pathological examination showed that form-like cells were observed only in the control group, suggesting the existence of proliferating pre-adipocytes only in the control group. Lipid-related plasma profiles showed no statistical differences. Dietary polyphenol did not induce any anorectic effects as reported in studies concerning tea polyphenol. We conclude that dietary APP has an anti-adipogenic effect in Wistar rats without any anorectic phenomenon.

Key words: apple, catechin, obesity, polyphenol

Introduction

Polyphenols provide a C6-C3-C6 backbone structure and are ubiquitous in the plant kingdom. Polyphenols, especially tea catechins, have been reported to lower plasma lipids and inhibit accumulation of adipose tissues [10, 11, 14, 15]. Murase et al. [16] showed that long-term consumption of tea catechins suppressed diet-induced obesity. They also showed that tea catechin induced significant increases of acyl-CoA oxidase and medium chain acyl-CoA dehydrogenase mRNA expressions as well as high $\beta$-oxidation activity in the liver. Dulloo et al. [4, 5] also showed that ingestion of tea catechins and caffeine mixture stimulated $O_2$ consumption and energy expenditure. They also confirmed that a catechin and caffeine mixture elevated the plasma
noradrenalin level. Dietary catechin was also reported to have an anorectic effect causing loss of adipose tissues [10, 11]. These reports clearly showed that tea catechin had a beneficial effect on reducing adipose tissue.

Apples represent a major proportion of the fruit supply. Apple polyphenols (APP) contain procyanidine (proanthocyanidine) as a major component [8, 9, 18, 21, 26]. Procyanidine consists of (+)-catechins and (-)-epicatechin units, which are widely found as secondary metabolites in plants [8, 26]. APP and purified procyanidine have various functionalities as antioxidants [6, 12, 13, 19, 20] and modulate the immune function [9]. As shown by the chemical structures, the biological functions of APP are quite similar to those of tea catechins. Regarding adipose tissue loss, in vitro cell culture studies showed that APP suppressed adipose cell formation [21]. Aprikian et al. [3] reported that dietary apple polyphenol, combined with pectin, lowered rat plasma cholesterol. Although previous reports suggested that dietary APP reduces adipose tissues weight [3, 21], a direct relationship between dietary APP and adipose tissue weight in vivo has not yet been fully examined.

Recently, metabolic syndromes such as diabetes have come to be regarded as serious diseases and approximately 60% of the people with metabolic syndromes have obesity as a contributing factor [17]. Hence, in order to prevent metabolic syndromes, it is very important to determine whether dietary polyphenols have beneficial effects in reducing the adipose tissues in vivo. To date, many obese rodent models created by controlling environmental factors (e.g. high fat diet) or by inducing spontaneous mutations (e.g. ob/ob mice) and genetically engineered animals have provided useful information on human obesity [23]. The application of APP as a biologically active additive in the case of experimental animals will provide valuable information not only on rat lipid metabolism but also on the prevention of human obesity. In this study, we hypothesized that dietary APP would have some effects on in vivo adipose tissue weight and analyzed the pathological features of fat pads and plasma characteristics to further evaluate the effects of dietary APP in vivo. Although some studies on dietary tea catechins have employed a high-fat diet as the control [16], in this study, we decided to use the ordinary rodent diet of AIN93M [2] as the control diet. The purpose of this was to focus on the normal dietary condition and to limit the biologically active additives to dietary APP.

Materials and Methods

All procedures used in this study were approved by the Ethical Committee of Nippon Sport Science University.

Apple polyphenol

Apple polyphenols (APP) were extracted from unripe apples (Malus pumila cv. Fuji). APP is a mixture of polyphenols mainly consisting of dimers to pentadecamers of procyanidins (about 45% w/w). Other components are as follows, phenolic acids (about 25% w/w; mainly chlorogenic acid), phloretin glycosides (about 10% w/w; mainly phloridizin), monomeric flavan-3-ols (about 15% w/w; catechin), and other compounds (about 5% w/w; mainly, quercetin glycosides) [9]. This composition is typical in natural apple polyphenols.

Animals and diets

Male Wistar rats obtained from CLEA Japan Inc. (Tokyo, Japan) at 10 weeks of age were maintained under a 12 h light-dark cycle. The animals were fed laboratory chow (CE-7, Clea, Japan) for 1 week. Rats were then divided into three diet groups: the 5% apple polyphenol (5%APP) group (N=8), the 0.5% apple polyphenol (0.5%APP) group (N=8) and the control (Con) group (N=8). To prevent variation between the groups, we segregated all the animals in order to maintain similar average body weights. Compositions of the diet of each group are listed in Table 1 (2). Animals were maintained on the diets for 3 weeks. Food was given to the animal in a dry form. The weight of each animal was recorded once a week throughout the experimental period.

After the experimental period, animals were anesthetized by an intraperitoneal injection of sodium pentobarbital (10 mg/100 g of body weight) and exsanguinated. The blood was collected and centrifuged to obtain serum. Immediately after sacrifice, the retroperitoneal, mesenteric and epididymal adipose tissues were removed. After measuring their weights, retroperitoneal adipose tissues were rapidly immersed in fixative as described below.
**Table 1.** Compositions of designed diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>0.5%APP</th>
<th>5%APP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>14.0</td>
<td>14.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>62.1</td>
<td>62.1</td>
<td>62.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td><em>Mineral mixture</em></td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Vitamin mixture</em></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>L-cystine</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Choline (bitartrate)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>t-butylhydroquinone</td>
<td>0.0008</td>
<td>0.0008</td>
<td>0.0008</td>
</tr>
<tr>
<td>APP</td>
<td>0</td>
<td>0.5</td>
<td>5</td>
</tr>
</tbody>
</table>

*AIN Mineral mixture and *AIN Vitamin mixture were obtained from Oriental Yeast Co., Tokyo, Japan. Composition of control diet is based on AIN93M which was reported by American Institute of Nutrition. Other diets were modified from control diet. APP: apple polyphenols.

**Pathological analysis**

The retroperitoneal adipose tissues were chemically fixed, embedded in paraffin blocks and sectioned to evaluate the pathological characteristics as described below. Retroperitoneal adipose tissues were soaked in 10% formaldehyde for fixation for more than 24 h at room temperature. After the fixative treatment, the tissues were dehydrated and mounted in paraffin blocks. Thin sections (4 μm) were obtained and stained with hematoxylin and eosin. The sections were examined by light microscopy (BX60 Olympus Japan) and the diameters of mature adipocytes were measured. Since we obtained 2-dimensional images, the average value of the longest and shortest inner diameters were determined and used as the estimated diameters. We measured one hundred cells in the 5%APP and the Con groups.

**Blood analysis**

The total cholesterol (cholesterol oxidase-DAOS method), triglyceride (GPO-DAOS method) and free fatty acid (ACS-ACOD method) contents were measured using the following commercially available kits: total cholesterol, cholesterol E kit; triglyceride, triglyceride C kit; free fatty acid, NEFA C kit (Wako, Tokyo, Japan). Plasma leptin was measured with an enzyme linked immuno enzyme assay (ELISA) using a commercially available kit (Yanaihara Laboratory, Tokyo, Japan).

**Statistical analysis**

All values are presented as means and standard deviation. Statistical comparisons among the three groups were made by one-way ANOVA with Bonferroni’s *post hoc* test. The un-paired two-tailed Student’s *t*-test was also used when two groups were compared. Significance of difference was accepted at the 5% level. Statistical analyses were carried out using SPSS Base system 10.1J for Windows (SPSS Japan, Inc., Japan).

**Results**

**Body weight and food intake**

Cumulative body weight change and food intake are shown in Fig. 1a and 1b. Average body weight among the three groups did not show any significant difference. Total and average energy intake of the 0.5% APP group was significantly higher than those of the other two groups (Fig. 1b and Table 2).

**Adipose tissue weight**

After the three-week experimental period, retroperitoneal, mesenteric and epididymal adipose tissues weights were measured. As shown in Table 2, the average weight of adipose tissues declined with increasing dose of APP. Retroperitoneal and epididymal adipose tissues weights of the 5%APP group were significantly lower than those of the control.

**Estimated diameters of adipocytes**

As shown in Fig. 2a and 2b, the diameter of the 5% APP was larger than that of the control. Statistical significance was observed between groups (*P*=0.0101). In addition, form-like cells were frequently seen only in the control group suggesting that actively proliferating pre-adipocytes existed in this group (Fig. 2a).

**Plasma analysis**

As shown in Table 3, the examined lipid-related parameters did not show any significant differences. Average values showed relatively higher concentrations of these materials in the APP groups in comparison with the control. We also measured leptin, which is generally known as a peptide hormone that plays a critical role in the regulation of body weight by inhibiting...
We confirmed that the plasma leptin level in the 5%APP group was significantly lower than those in the other two groups.

Discussion

The effects of polyphenols on adipose tissue formation have previously been reported. Especially in tea catechin, activation of lipid metabolism was clearly shown by some researchers [5, 16]. In this study, we hypothesized that dietary apple polyphenols would also have beneficial effects on preventing obesity. Finally, we found that dietary APP groups showed significantly lower adipose tissue weights. retroperitoneal adipose tissue weight in the 5%APP group was about 20% lower than that of the control.
Other adipose tissues such as mesenteric and epididymal adipose tissues showed the same tendency. Murase et al. showed that tea catechins reduced retroperitoneal white adipose tissues as follows: 0.5% catechin diet, 25% loss of adipose tissues; 0.2% diet, 51% loss; and 0.1%, 86% loss [16]. It should be noted that Murase et al. employed a high (30%) fat diet. Such a condition highlights the beneficial effect of polyphenols on dietary induced obesity. We employed young adult (10 weeks of age) rats and a normal diet (4% fat based on AIN93M) to reproduce a normal or sport-like situation and to eliminate factors other than dietary APP. Despite the relatively low, but not uncommon level of dietary fat used in this study, the same tendency toward low adipose tissue was confirmed. The experimental period and species are other points to consider. Murase et al. examined mice for 11 months while our trial lasted 3 weeks using rats. Our experimental period is considered to replicate a supplemental diet prior to some competition, for example. Employment of the same experimental conditions would be needed for direct comparison, but dietary APP also appeared to have effective for adipose tissue loss.

Kao et al. [10, 11] showed that epigallocatechin gallate (EGCG), which is a representative polyphenol found in tea, had anorectic properties effecting a loss in body weight. Murase et al. [16] also showed that the energy intake of the 0.5% dietary catechin groups was 5.6% lower than that of the control. These reports suggest that the anorectic properties of tea catechin, especially EGCG, partially contribute to adipose tissue loss. In this study, we found that the initial calorie intake of the 5%APP group was lower than that of the control. Our findings partially match those of previous studies. The catechin investigated in our study, procyanidin, contains multimeric forms of catechins. The chemically different structures might cause different energy intake behaviors. In fact, Kao et al. have shown that the anorectic property was catechin specific. Further, EGCC–but not the other green tea polyphenols such as epicatechin (EC), epigallocatechin (EGC) or epicatechin gallate (ECG)–resulted in the reduction of food intake in Sprague-Dawley rats [10]. Since anorectic proper-

![Graph](image-url)
ties are not always welcomed, APP should be a better dietary aid for weight loss than tea catechin.

We estimated the diameters of mature adipocytes to partially investigate the mechanisms of APP’s anti-obesity effect. Unexpectedly, we confirmed that the average diameter of mature adipocytes in the 5%APP group was larger than that of the control. It has been hypothesized that an increase in the number of fat cells in adults does not occur until the existing fat cells reach a critical size [7]. Since Harmelen et al. confirmed that the fat cell volume did not further increase in an obese subgroup (body mass index (BMI): 25–29.9 kg/m²) when compared to an overweight group (BMI >30 kg/m²), they concluded that increase in fat cell size apparently precedes the increase in fat cell number in humans [25]. Sugihara et al. examined the proliferation ability of adipocytes matured in vitro [22]. They showed that the unilocular fat cell in Ham F12 medium supplemented with 10% newborn calf serum became multilocular fat cells and fibroblast-like fat cells, which could divide and differentiate to unilocular fat cells again. If in vivo mature adipocytes divide themselves as observed in vitro by Sugihara et al., their diameter would become smaller and multilocular fat cells would appear. The results of these previous studies lead us to conclude that dietary 5% APP prevents hyperplasia but not hypertrophy of fat cells. This adipocytes of the 5%APP group became larger, but on the other hand, adipocytes of the control fully enlarge and divide themselves to further proliferation, and consequently, the apparent diameter of the control adipocytes appeared smaller.

We analyzed plasma characteristics related to fat metabolism. As a result, we confirmed that the plasma profiles showed no significant differences between any two groups, except for leptin. These results suggest that dietary APP has a lesser effect on lipolysis than adipogenesis. Care must be taken, since we measured these parameters in a non-fasted condition. All data were probably affected by the food ingestion just before blood collection. Detailed average energy intakes on the last day in the control, 0.5%APP and 5%APP groups were 7.5 kcal/day/rat, 9.4 kcal/day/rat and 8.5 kcal/day/rat, respectively, as shown in Fig. 1b. Murase et al. also evaluated the plasma characteristics under non-fasting conditions. Their data suggests an association between the plasma profiles of fat and energy consumption. They also showed that the total cholesterol of rats that were fed with tea cathechins was significantly lower than that of the controls. Thus, it is necessary to consider that energy consumption might be associated with plasma profiles and hence, evaluation under fasting conditions should provide important information.

Careful attention should be paid to the significantly low body weight of the 5%APP group regardless of the similarity in energy consumption. This tendency was maintained in the 0.5%APP group since the body weight of the 0.5%APP group was similar to that of the control regardless of the significantly higher energy intake. Although we have shown that dietary APP inhibits adipogenesis even under energy-rich conditions, the detailed molecular mechanisms are still unclear. We intend to evaluate the same experiment under pair-fed conditions to further determine the effects of dietary APP on adipogenesis, particularly under low APP concentrations.

Unlike other plasma materials, the leptin level of the 5%APP group was significantly lower than that of the control. Regardless of fasted or non-fasted state, Murase et al. showed that the plasma leptin level of the 0.5% dietary tea catechin group was significantly lower (non-fasting condition, 5.1 ± 3.2 ng/ml; fasting condition, 0.5 ± 0.5 ng/ml) than that of the high-fat control (non-fasting condition, 54.7 ± 11.4 ng/ml; fasting condition, 18.7 ± 9.5 ng/ml) [16]. Although orally administered foods should also affect the leptin level, their effect might not be so apparent on leptin. A significantly lower level of leptin in the 5%APP group matched the finding of Murase et al. and suggests that the plasma leptin level tends to correlate with body fat content as stated by others [1]. We also consider that the association between the plasma leptin level and body fat content tended to maintain a lower plasma leptin level in the 5%APP group under the non-fasting conditions.

We conclude that dietary APP has an inhibitory effect on adipose tissue formation in Wistar rats. Since APP does not contain caffeine, we can further analyze the molecular mechanisms underlying the physiologic outcome. We also emphasize that dietary APP did not show any anorectic effect. It is important that a weight loss agent does not cause any side effects, such as eating disorder. We believe that these results could be extrapolated to human obesity, as has been done in
the case of many animal obese models. Based on this, APP might be a beneficial diet component that is devoid of any apparent side effects for the prevention of metabolic syndromes in humans.

Acknowledgment

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References