

Effects of Dietary Procyanidins and Tea Polyphenols on Adipose Tissue Mass and Fatty Acid Metabolism in Rats on a High Fat Diet

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Edited by K. Miyashita, Hokkaido Univ., and accepted November 4, 2005 (received for review August 11, 2005)

Abstract: Large amounts of procyanidins, made up of catechin oligomers, are consumed on a daily basis via various plant-based diets. Recent studies show that tea polyphenol (TP) exerts anti-obesity effect; however, the biological functions of procyanidins have not yet been established. In this study, we examined the high dose effects of procyanidins from apple polyphenol (AP), hop polyphenol (HP), and tea polyphenol (TP) on obesity using normal Sprague-Dawley rats on a high fat (HF) diet. We measured adipose tissue mass and tissue lipid parameters in rats on polyphenol-free low fat (LF) (7%) or HF (27.8%) diets with or without 1% of each polyphenol for 9 weeks. Dietary AP, HP, and TP suppressed the increase in white adipose tissue. Dietary TP increased the level of fatty acids excreted into feces; however, this was not observed in the AP- or HP-fed group. Each dietary polyphenol tended to reduce fatty acid synthesis and promote fatty acid β -oxidation as compared with a HF diet alone. Moreover, each dietary polyphenol tended to increase the level of peroxisome proliferator-activated receptor alpha (PPAR α) in the liver and decreased the levels of PPAR γ in brown and white adipose tissues. Therefore, we speculate that procyanidins from AP or HP may reduce the increase in white adipose tissue induced by an HF diet through a combination of the agonist-like action of PPAR α and antagonist-like action of PPAR γ . On the other hand, TP may exert an anti-obesity effect via the combined effect of PPAR α and PPAR γ described above as well as the promotion of fatty acid excretion into feces.

Key words: catechin, lipid metabolism, obesity, procyanidin

1 Introduction

The intimate relationship between the risk of incidence of various lifestyle-related diseases, such as type II diabetes and coronary heart disease, and obesity has been well recognized. In obesity, as compared with subcutaneous adipose accumulation, visceral adipose accu-

mulation contributes more strongly to the incidence of various lifestyle related diseases. Therefore, the prevention or amelioration of obesity by dietary manipulation can reduce the risk factors of lifestyle-related diseases. Some studies reported that dietary polyphenols, such as tea catechins (1,2) exerted an anti-obesity effect in obese rodent models on a high fat (HF) diet; however,

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their mechanisms of action remain ambiguous. Moreover, large amounts of polyphenols are consumed on a daily basis via various plant-based diets. In spite of this, the modulating effect of procyanidins, on lipid metabolism including their anti-obesity effect has not been elucidated well. Procyanidins are composed of catechin oligomers (3). **Figure 1** shows their structures. In a recent study, absorption of dimeric procyanidins from cocoa was observed (4). Some studies showed that procyanidins exhibited various biological activities, including radical scavenging (5), antibacterial (6), antiviral (7), anticarcinogenic (8), antiinflammatory (9), and vasodilating (10) activities. Recent studies showed that a high dose of tea catechins exerted an anti-obesity effect by modulation of lipid metabolism in diabetic mice models on a HF diet (1). Therefore, it is possible that dietary procyanidins also exert an anti-obesity effect because they are oligomers of (–)-epicatechin as shown in **Fig. 1**. In this study, we examined the high dose effect of procyanidins from apple polyphenol (AP) (approximately 64% of procyanidins composed of 2-14-mer (–)-epicatechins and major procyanidins are 2 and 3 mer), hop polyphenol (HP) (approximately 60% of procyanidins composed of (–)-epicatechins more than 8 mer), and tea polyphenol (TP) composed monomeric catechins, in order to evaluate the difference

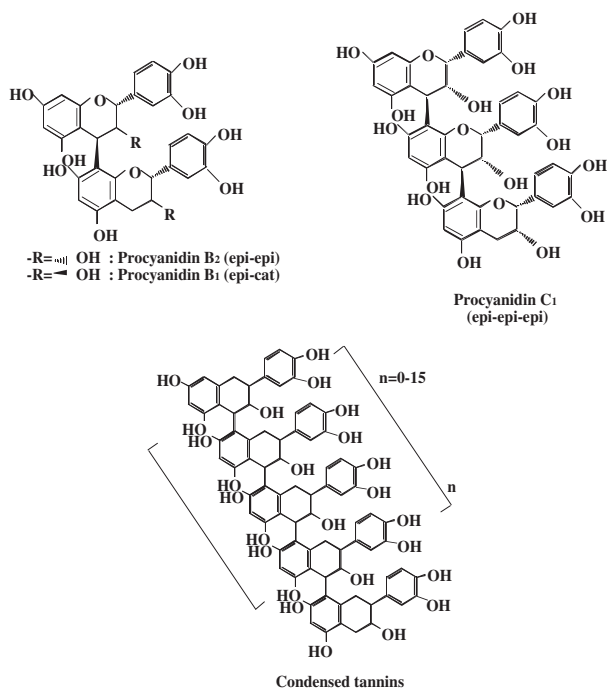


Fig. 1 Structures of Procyanidin Compounds.

of their biological functions as inhibitors of obesity induced in normal rats by the consumption of a HF diet.

2 Experimental

2.1 Polyphenols

The composition of each dietary polyphenol is shown in **Table 1**. TP is a caffeine-free powder and is composed of polyphenol monomers. AP have approximately 64% of procyanidins composed of 2-14-mer (–)-epicatechins. HP contain approximately 60% of procyanidins composed of (–)-epicatechins more than 8 mer. These pure polyphenols were gave from Asahi Brewery Co., Ltd. (Tokyo, Japan).

2.2 Animals and Diets

Sprague-Dawley rats (3-week-old males, CLEA Japan, Co., Ltd. Tokyo, Japan) were housed individually in a room with controlled temperature (20–23°C) and light (08:00 to 20:00) conditions. After the rats were acclimated for 1 week, they were divided into five groups (each six rats) and maintained on the following diets (**Table 2**): low fat diet (LF) (7% soybean oil), HF diet (7% soybean oil and 20.8% lard), HF + 1% TP diet (HF + TP), HF + 1% AP diet (HF + AP), and HF + 1% HP diet (HF + HP). Each diet was prepared according to AIN93 recommendations (11). In the groups that were fed a HF diet with polyphenols, the quantity of the diet was the same as that consumed by the LF and HF groups under the pair-feeding condition because dietary polyphenols sometimes caused a slight reduction in food intake. After 9 weeks, rats were lightly anesthetized using diethylether. They were then bled from the abdominal aorta and various tissues were quickly excised. Plasma was prepared by centrifugation after allowing blood to clot at room temperature. The samples were maintained at –80°C until analyses. The animal experiments were conducted according to the guidelines provided by the ethical committee of experimental animal care at Hirosaki University.

2.3 Measurement of Plasma Levels of Insulin, Leptin, and Adiponectin

The levels of plasma insulin, leptin, and adiponectin were measured by ELISA by using a commercial kit (Rat Insulin ELISA kit: Mercodia, Uppsala, Sweden; Mouse/Rat Adiponectin ELISA kit: Otsuka Pharmacol. Ltd, Tokyo, Japan; Rat Leptin ELISA YK 050: Yanai-

Table 1 Composition of Dietary Polyphenols.

Componet	Dietary polyphenols		
	Tea polyphenol	Apple polyphenol	Hop polyphenol
	weight%		
Epicatechin	9.0		
Epigallocatechin	11.0		
Epicatechine gallate	11.0		
Epigallocatechin gallate	57.0		
Other catechines	3.0		
Procyanidin 2mer		11.1	
Procyanidin 3mer		12.3	
Procyanidin 4mer		8.7	
Procyanidin 5mer		5.9	
Procyanidin 6mer		4.9	
Procyanidin >7mer		20.9	
Procyanidin >8mer			60.0
Other flavonoids		18.9	15.0
Astragaline			3.0
Isoquercitlin			2.0
Others	9.0	15.1	20.0

hara Institute Inc., Shizuoka, Japan, respectively).

2·4 Serum and Liver Lipid Analyses

Liver and serum lipids were extracted by the method of Folch *et al.* (12), and the levels of liver and serum lipids were measured as described previously (13).

2·5 Fecal Fatty Acid Analysis

The levels of excreted fatty acids in each group were analyzed according to the method of Van de Kamer (14).

2·6 Preparation of Liver Mitochondria/Peroxisomes

A piece of liver (approximately 3 g) was homogenized in 7 volumes of ice-cold 0.25 M sucrose homogenate solution containing 1 mM EDTA and 3 mM Tris-HCl (pH 7.2) buffer. The homogenate was centrifuged at $500 \times g$ for 10 min at 4°C. The supernatant was recentrifuged at $9,000 \times g$ for 10 min at 4°C to isolate mitochondria. Mitochondria were washed twice with the homogenate solution and finally suspended in the same solution. The $9,000 \times g$ supernatant was centrifuged at $105,000 \times g$ for 60 min at 4°C to obtain a sediment of microsomes; the remaining supernatant was used as the cytosolic fraction.

2·7 Assays of Enzyme Activity

Fatty acid synthase activity was determined spectrometrically by the method of Neporkroeff *et al.* (15). The reaction solution was 0.2 M potassium phosphate buffer (pH 7.0) containing 0.4 mM EDTA, 200 μ M malonyl-CoA, 50 μ M acetyl-CoA, 300 μ M NADPH, and the sample suspension. One unit of fatty acid synthase is defined as the amount of enzyme protein

Table 2 Composition of the Diets.

Component	Group				
	LF	HF	HF+TP	HF+AP	HF+HP
	weight%				
Cornstarch	34.8	—	—	—	—
Casein	23.6	23.6	23.6	23.6	23.6
α -Cornstarch	13.2	8.4	7.4	7.4	7.4
Sucrose	10	28.8	28.8	28.8	28.8
Soybean oil	7	7	7	7	7
Lard	—	20.8	20.8	20.8	20.8
Cellulose	6.4	6.4	6.4	6.4	6.4
Mineral mix (AIN93)	3.5	3.5	3.5	3.5	3.5
Vitamin mix (AIN93)	1	1	1	1	1
L-Cystine	0.3	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2	0.2
Polyphenol	—	—	1	1	1

LF, low fat diet; HF, high fat diet; TP, tea polyphenol; AP, apple polyphenol; HP, hop polyphenol.

required to synthesize 1 nmol of palmitic acid (equivalent to the oxidation of 14 nmol of NADPH) per min at 30°C. Glucose-6-phosphate dehydrogenase (G6PDH: EC1.1.1.49) activity was measured according to the method of Dror *et al.* (16). The reaction mixture (pH 7.6) contained 0.1 M Tris-HCl, 30 mM MgCl₂, 3.3 mM glucose-6-phosphate, 1.2 mM NADP, 0.5 units/ml 6-phosphogluconate dehydrogenase, and the sample suspension. One unit of G6PDH activity was expressed as 1 nmol production of NADPH per min. The peroxisomal acyl-CoA oxidase activity was assayed by measuring the palmitoyl-CoA-dependent H₂O₂ production at 30°C by the method of Allain *et al.* (17). The reaction mixture contained 58 mM potassium phosphate (pH 7.4), 10.6 mM phenol, 0.82 mM 4-aminoantipyrine, 10 mM FAD, 4 U horse radish peroxidase, 0.1 mM palmitoyl-CoA, 0.2 mg/ml of albumin, and the enzyme solution.

2·8 Western Blot Analysis of Tissue Peroxisome Proliferator-Activated Receptors

Tissue lysates from adipose tissues and liver were prepared by lysis using the CellLytic MT mammalian tissue lysis/extraction reagent (Sigma Chemicals, St. Louis, MO). Protein concentration was measured using the Bio-Rad protein assay kit (Bio-Rad Laboratories Co. Ltd., Hercules, CA). Protein extracts at the same concentration were separated on a 12% SDS-PAGE gel. The gel was transblotted onto a PVDF membrane which was blocked with a blocking buffer (0.5% skim milk in PBS containing 0.1% Tween 20) overnight. The transblotted membranes were then incubated with a rabbit polyclonal anti-peroxisome proliferator-activated receptor alpha (PPAR α , 1:500 v/v dilution, Cayman Chemical Co., Ann Arbor, MI) or rabbit polyclonal anti-PPAR γ antibody (1:500 v/v dilution, Affinity Bioreagents Ltd., Golden, CO) for 2 h. The membranes were then washed with the blocking solution and incubated with a secondary antibody (anti-goat IgG conjugated to horseradish peroxidase, 1:10,000 v/v dilution) for 1 h. After washing with the blocking solution, the signal was detected with a chemiluminescence kit (ECLplus kits Amersham, Arlington Heights, IL). Films were analyzed by densitometry using the NIH image software and the fold increase or decrease is reported in the figures. Each signal of the LF group is considered as 1.

2·9 Statistical Analyses

All values are presented as mean \pm standard error. Statistical comparisons between groups were analyzed by Duncan's new multiple-range test (18) and the Super ANOVA software. Statistical significance was defined as $p < 0.05$.

3 Results

3·1 Effect of Dietary Polyphenols on Growth Parameters

The weight gain in the HF group was significantly higher than that in the LF group (Table 3). None of the dietary polyphenols affected weight gain in the rats on the HF diet. Compared with the LF diet, the HF diet significantly increased liver weight even when rats were fed AP or HP; however, dietary TP tended to reduce the increase in liver weight (HF vs. HF + TP, $p = 0.053$; HF + TP vs. HF + AP, $p = 0.055$; HF + TP vs. HF + HP, $p = 0.0492$). The weight of epididymal (HF vs. HF + each $p = 0.3057 \sim 0.3641$) and perirenal (HF vs. HF + each $p = 0.1077 \sim 0.1608$) white adipose tissues tended to decrease in rats on the HF diet with polyphenols compared with those on the HF diet alone; however, these effects were not statistically significant. The weight of mesenteric adipose tissue in rats that were fed each type of polyphenol was significantly lower than in those of the HF group, particularly of the HF + AP group. Therefore, the total white adipose tissue levels in rats on the HF diet with polyphenols were significantly lower than in those on the HF diet alone.

3·2 Effect of Dietary Polyphenols on Plasma Adiponectin, Leptin, and Insulin Levels

Compared with the LF diet, the consumption of the HF diet increased the plasma leptin level; however, dietary polyphenols tended to reduce these levels (HF vs. HF + TP, $p = 0.1030$; HF vs. HF + AP, $p = 0.0846$; HF vs. HF + HP, $p = 0.195$) in rats on the HF diet irrespective of the degree of catechin oligomerization (Fig. 2). The plasma adiponectin level was significantly lower in the HF group than in the LF group. This down-regulation of adiponectin secretion was not modulated by each dietary polyphenol. Moreover, dietary polyphenols tended to decrease the plasma insulin levels even when the rats on the HF diet; however, this effect was not statistically significant (HF vs. HF + each polyphenol).

Table 3 Effect of Dietary Polyphenols on Body Weight Gain, Liver Weight and Weight of White Adipose Tissues.

	Group				
	LF	HF	HF+TP	HF+AP	HF+HP
Food intake (g)	1105 ± 1	1103 ± 4	1101 ± 17	1101 ± 33	1100 ± 11
Initial body weight (g)	98.2 ± 2.1	98.3 ± 2.9	98.2 ± 4.6	98.2 ± 2.7	98.2 ± 3.4
Weight gain (g)	114.6 ± 3.4 ^a	163.5 ± 2.5 ^b	161.4 ± 3.3 ^b	164.8 ± 8.7 ^b	164.6 ± 3.0 ^b
Liver weight (g/100 g of body weight)	2.89 ± 0.03 ^a	3.20 ± 0.10 ^b	2.98 ± 0.09 ^{ab}	3.20 ± 0.11 ^b	3.20 ± 0.07 ^b
Epididymal WAT (g/100 g of body weight)	1.81 ± 0.15 ^a	2.55 ± 0.12 ^b	2.35 ± 0.12 ^b	2.32 ± 0.26 ^b	2.44 ± 0.13 ^b
Perirenal WAT (g/100 g of body weight)	2.10 ± 0.10 ^a	3.07 ± 0.28 ^b	2.66 ± 0.27 ^b	2.67 ± 0.15 ^b	2.60 ± 0.22 ^b
Mesenteric WAT (g/100 g of body weight)	1.03 ± 0.13 ^a	1.75 ± 0.18 ^b	1.20 ± 0.17 ^a	1.00 ± 0.12 ^a	1.29 ± 0.12 ^a
Total WAT (g/100 g of body weight)	4.94 ± 0.25 ^a	7.36 ± 0.46 ^b	6.21 ± 0.30 ^c	5.99 ± 0.39 ^c	6.34 ± 0.33 ^c

Each value is the mean ± SE (n=6). Values without a common superscript in a row are significantly different (P<0.05).

WAT, white adipose tissue; other abbreviations are same as in **Table 2**.

nol, $p = 0.3366 \sim 0.4405$).

3.3 Effect of Dietary Polyphenols on Hepatic Enzyme Activities

In our study, compared with the LF diet, the consumption of the HF diet lowered the activity of hepatic fatty acid synthase (**Fig. 3**). Each dietary polyphenol tended to decrease this enzyme activity in the HF group; however, the effects were not necessarily statistically significant (HF vs. HF + TP, $p = 0.1528$; HF vs. HF + AP, $p = 0.0761$; HF vs. HF + HP, $p = 0.0303$). The same tendency was observed with regard to the G6PDH activity; however, it was not statistically significant (HF vs. HF + each polyphenol, $p = 0.3615 \sim 0.4632$). The activity of peroxisomal β -oxidase (acyl-

CoA oxidase) also tended to decrease in the HF group as compared with the LF group. Contrary to this observation, dietary polyphenols, particularly dietary AP, tended to increase the activity of peroxisomal β -oxidase in rats on the HF diet (HF vs. HF + TP, $p = 0.2516$; HF vs. HF + AP, $p = 0.0702$; HF vs. HF + HP, $p = 0.4291$).

3.4 Effect of Dietary Polyphenols on Liver and Serum Lipid Levels

The lipid levels of the tissues were modulated by each dietary polyphenol. The serum triacylglycerol level was significantly higher in the HF group than in the LF group; however, each dietary polyphenol ameliorated the increase in the triacylglycerol level induced by the consumption of the HF diet, particularly in the

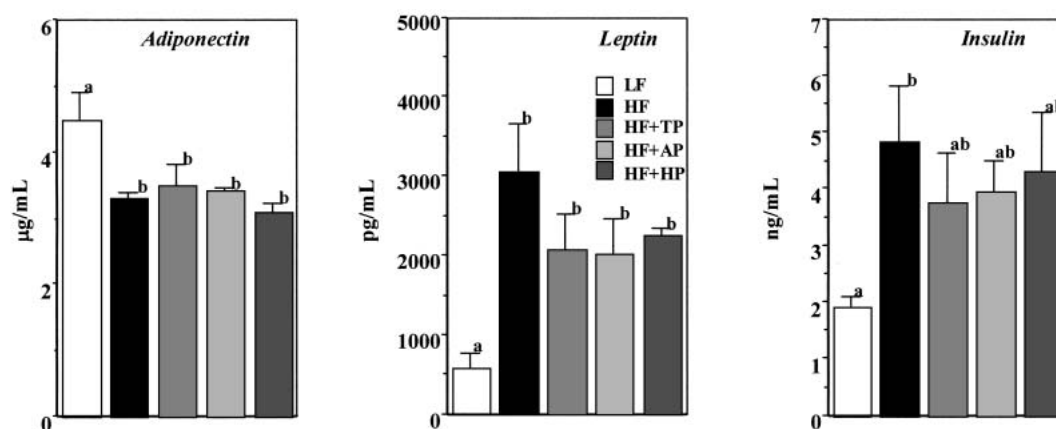


Fig. 2 Effect of Dietary Polyphenols on Serum Adiponectin, Leptin, and Insulin Levels of Rats Fed on a High Fat Diet.

Each value is the mean ± SE (n=6). Values without a common superscript in a row are significantly different. Abbreviations: LF, low fat diet; HF, high fat diet; TP, tea polyphenol; AP, apple polyphenol; HP, hop polyphenol.

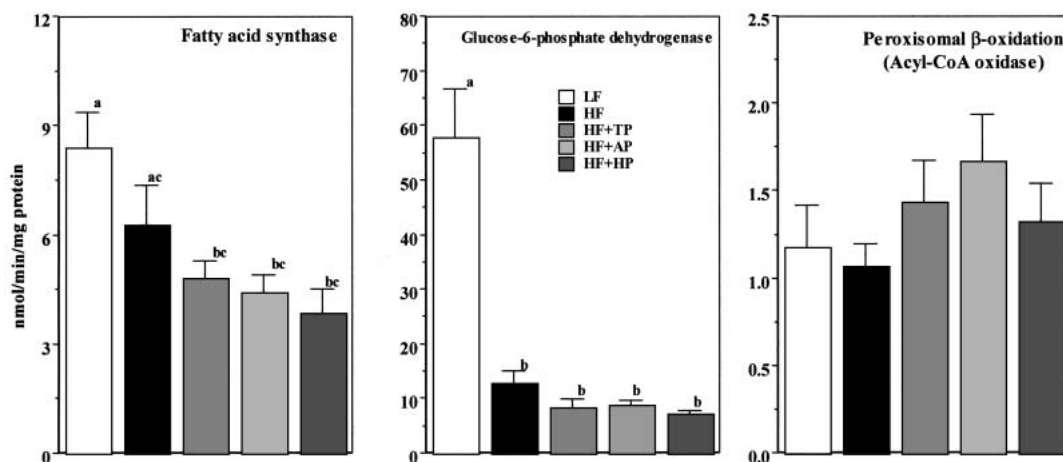


Fig. 3 Effect of Dietary Polyphenols on the Activities of Liver Fatty Acid Synthase, Glucose-6-phosphate dehydrogenase, and Peroxisomal β -Oxidation in Rats Fed on a High Fat Diet. Each value is the mean \pm SE (n=6). Values without a common superscript in a row are significantly different. Abbreviations: LF, low fat diet; HF, high fat diet; TP, tea polyphenol; AP, apple polyphenol; HP, hop polyphenol.

HF + TP group (Table 4). Dietary polyphenols also significantly lowered the levels of liver triacylglycerol even when the rats were on the HF diet. Both dietary TP and HP prevented the increase in the serum cholesterol level induced by the consumption of the HF diet; however, the preventive effect of dietary AP was not statistically significant. Moreover, the HDL-cholesterol level was significantly lower in the HF group than in the LF group; however, their levels in both the HF + TP and HF + AP groups were comparable to those in the LF group. As a result, the ratios of HDL-cholesterol to total cholesterol in the groups that were fed polyphenols were comparable to that of the LF group, although the HF diet alone decreased their ratio. Contrary to these observations, compared with the LF diet, the HF diet significantly increased the liver cholesterol level despite the consumption of polyphenols.

3.5 Effect of Dietary Polyphenols on Fatty Acid Excretion into Feces

As compared with the LF group, the amount of fatty acids excreted into feces in the HF group (104.5 ± 17.1 mg/day) was significantly higher than that in the LF group (36.7 ± 8.3 mg/day). Dietary TP significantly increased the amount of fatty acids excreted into feces (150.2 ± 18.7 mg/day) as compared with the HF diet alone; however, this was not observed in the AP group (114.3 ± 5.2 mg/day) and the HP group ($91.5 \pm$

17.1 mg/day).

3.6 Effect of Dietary Polyphenols on the Level of PPARs in the Adipose Tissue and Liver

The PPAR α protein level in the liver tended to be higher in the HF group than in the LF group (Fig. 4). Dietary polyphenols tended to increase the PPAR α levels as compared with the HF diet alone; however this effect was not statistically significant (HF vs. HF + each polyphenol, $p = 0.6776 \sim 0.7442$). Dietary polyphenols did not affect the PPAR α protein level in brown adipose tissue; however, the protein level in the HF + AP group tended to decrease as compared with that in the HF group ($P = 0.0627$). On the other hand, the protein level of PPAR γ in brown adipose tissue tended to be lower in rats that were fed polyphenols, particularly in the HF + AP group (HF vs. HF + TP, $p = 0.2472$; HF vs. HF + AP, $p = 0.017$; HF vs. HF + HP, $p = 0.136$). Dietary TP and AP significantly reduced the PPAR γ protein level in white adipose tissue as compared with the LF diet.

4 Discussion

We examined the high dose effect of either AP, HP, or TP on growth parameters in normal rats in which obesity was induced by the consumption of a HF diet in

Table 4 Effect of Dietary Polyphenols on Tissue Lipid Levels in the Rats Fed High Fat Diet.

	Group				
	LF	HF	HF+TP	HF+AP	HF+HP
Serum					
Triacylglycerol (mg/dl)	58.1 ± 7.9 ^c	158.8 ± 30.5 ^b	75.7 ± 8.1 ^a	97.8 ± 15.8 ^a	99.8 ± 12.6 ^a
Cholesterol (mg/dl)	56.8 ± 5.1 ^{ac}	75.7 ± 4.1 ^b	54.4 ± 3.6 ^{ac}	67.4 ± 2.9 ^{bc}	56.2 ± 2.6 ^{ac}
HDL-cholesterol (mg/dl)	29.8 ± 3.8 ^a	20.3 ± 3.8 ^b	27.0 ± 1.2 ^{ab}	28.5 ± 3.1 ^{ab}	21.0 ± 1.2 ^{ab}
Phospholipids (mg/dl)	89.2 ± 6.5 ^a	93.4 ± 8.8 ^a	52.3 ± 4.2 ^b	60.8 ± 5.3 ^b	46.3 ± 6.2 ^b
Liver					
Triacylglycerol (mg/g)	33.9 ± 3.5 ^a	82.7 ± 2.2 ^b	64.3 ± 11.5 ^c	67.0 ± 3.4 ^c	65.2 ± 10.7 ^c
Cholesterol (mg/g)	24.4 ± 2.0 ^a	43.4 ± 3.1 ^b	38.9 ± 4.4 ^b	49.0 ± 3.4 ^b	48.6 ± 8.7 ^b
Phospholipids (mg/g)	3.35 ± 0.06 ^a	3.10 ± 0.06 ^b	3.23 ± 0.09 ^{ab}	3.14 ± 0.06 ^b	3.14 ± 0.05 ^b

Each value is the mean ± SE (n=6). Values without a common superscript in a row are significantly different (P<0.05).

HDL, high density lipoprotein; other abbreviations are the same as in **Table 2** and **Table 3**.

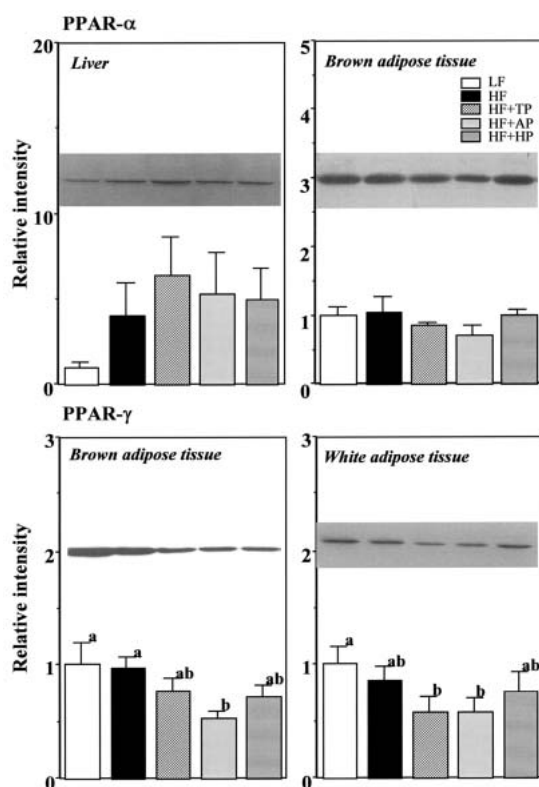


Fig. 4 Effect of Dietary Polyphenols on the Levels of Peroxisome Proliferator-activated Receptors (PPAR α and PPAR γ) in Rats Fed on a High Fat Diet.

See the detailed procedures in the text. Each value is the mean ± SE (n=6). Values without a common superscript in a row are significantly different. Abbreviations: LF, low fat diet; HF, high fat diet; TP, tea polyphenol; AP, apple polyphenol; HP, hop polyphenol.

order to evaluate the anti-obesity effects of procyanidins from AP, HP, and TP. Each polyphenol tended to prevent the increase in adipose tissue mass induced by the intake of the HF diet, although we did not observe modulation of hepatic function by a high dose of polyphenols (data not shown). Dietary powdered green tea suppressed body weight increase and various adipose tissue weights in male Zucker rats on the HF diet (19). The high dose of purified tea catechins also significantly reduced body weight gain and fat accumulation in the C57BL/6J mice on the HF diet (1). Thus, a high dose of monomeric polyphenols, such as tea catechins, may exhibit an anti-obesity effect accompanied with a significant reduction in body weight in obese rodent models. In our experiment using normal rats, weight gain was not altered by the consumption of each polyphenol; however, the accumulation of white adipose tissue induced by the consumption of the HF diet was ameliorated by the consumption of each polyphenol. These contradictions may be ascribed to modulation in the development of the muscular system because we recently observed that dietary AP increased the excreted level of creatinine into urine in the OLETF rat (unpublished data: polyphenol-free, 9.09 ± 0.40 mg/dl; AP, 10.76 ± 0.58 mg/dl; condensed tannin, 9.59 ± 0.26 mg/dl). Therefore, the effect of dietary procyanidins on muscle development will be examined in a future study.

It is well recognized that the physiologically active substances called adipocytokines in recent advanced obese studies. Leptin, which is an obese gene product, is secreted in excess levels from the enlarged adipose tissues of the obese and plays an important role in regu-

lating the size of the adipose tissue mass (20). Adiponectin is the major secretory protein produced specifically by the adipose tissue and its levels are known to be reduced in obesity (21). The HF diet induced an increase in the plasma leptin level as compared with the LF diet; however, each dietary polyphenol, irrespective of its procyanidin composition, significantly reduced the plasma leptin levels even when rats were on the HF diet (Fig. 2). The same observations were found in C57BL/6J mice in which obesity was induced by the consumption of the HF diet with tea catechin 1 or purple corn color (22). Therefore, dietary procyanidins as well as catechins or anthocyanins, may also exert a modulating effect on leptin secretion. It is possible that the inhibition of white adipose tissue accumulation partly contributes to the decrease in plasma leptin because it may simply be a marker of the adipose tissue mass. The plasma adiponectin level was significantly lower in the HF group than in the LF group. This downregulation of adiponectin secretion was not modulated by each dietary polyphenol. The long-term consumption of oolong tea increased the plasma adiponectin level in patients with coronary artery disease (23); however, it is uncertain whether polyphenolic compounds really affect this secretion because the long-term consumption of caffeine present in oolong tea significantly reduced body weight (24). Therefore, it is possible that our use of TP without caffeine did not produce downregulation of adiponectin secretion from adipocytes. Similar to that observed in the case of leptin levels, dietary polyphenols tended to decrease the plasma insulin levels even when the rats were fed the HF diet, although adiponectin expression correlates with insulin sensitivity (25). The inhibitory action of dietary polyphenols on the accumulation of white adipose tissues may contribute to the decrease in plasma insulin levels as shown in other studies (1,26).

The high dose of cyanidin 3-glucoside-rich purple corn color exerts an anti-obesity effect in the C57BL/6J mice liver through the inhibition of mRNA expression of lipogenic enzymes, including fatty acid synthase, acyl-CoA synthase, and glycerol-3-phosphate acyltransferase (22). Similarly in the present study, the activity of hepatic fatty acid synthesis appears to be lowered by the consumption of the HF diet with each dietary polyphenol as compared with the HF diet alone (Fig. 3). The long-term consumption of a high level of tea catechins significantly increased acyl-CoA oxidase and

medium chain acyl-CoA dehydrogenase mRNA expression as well as β -oxidation in the C57BL/6J mice liver (1). The downregulation of fatty acid synthesis or the upregulation of fatty acid β -oxidation may contribute to the inhibition of white adipose tissue accumulation. Based on our results, it is possible that dietary procyanidins such as apple polyphenols that have a high level of catechin dimers and trimers may promote β -oxidation of fatty acids.

In this experiment, dietary AP or HP as well as TP, exerted a hypolipidemic effect. The same observation was reported in studies using obese rodent models that were fed tea catechin (1) or hamsters that were fed a green tea extract (27). The former study relates the hyperlipidemic effect of tea catechins to the modulation of lipid metabolism. However, the latter suggests that the hypolipidemic action of catechins is most likely mediated by inhibiting dietary fat absorption. It is possible that the hypolipidemic action of dietary polyphenols can be ascribed to both the modulation of lipid metabolism and the inhibition of fat accumulation. In fact, we observed that dietary TP significantly increased the fecal excretion of fatty acids. Therefore, TP may exert a potent inhibitory action on intestinal fatty acid absorption through the decrease in the micellar solubility of fatty acids (28) or inhibition of lipase activity (29). Contrary to this action by TP, the intestinal absorption of fatty acids may not be influenced by AP or HP in rats that were on the HF diet.

Dietary polyphenols also significantly lowered the levels of liver triacylglycerol even when the rats were on the HF diet. These observations may be associated with the modulation of each enzyme activity that is related to the downregulation of fatty acid synthesis and the upregulation of β -oxidation by the consumption of polyphenols.

Compared with the LF diet, the consumption of the HF diet induced an increase in serum and liver cholesterol levels; however, we observed that the intake of polyphenols ameliorated the increase in serum cholesterol levels. Moreover, the atherogenic index (the ratio of (total cholesterol - HDL-cholesterol) to HDL-cholesterol) in groups that were fed polyphenols (HF + TP, 0.91 ± 0.12 ; HF + AP, 1.54 ± 0.37 ; HF + HP, 1.54 ± 0.13) was comparable to that of the LF group (1.07 ± 0.37); however, the cholesterol level in the HF group (4.13 ± 1.78) was the highest among all groups. Thus, dietary procyanidins as well as TP may exert an anti-

atherogenic effect through the modulation of lipoprotein metabolism. Contrary to these observations, the HF diet significantly increased the liver cholesterol level as compared with the LF diet, despite consumption of polyphenols. A high dose of lard may offset the lipid-lowering effect of dietary polyphenols because plasma cholesterol levels and hepatic cholesterol biosynthesis may increase in rats fed an excess of dietary saturated fatty acids (30).

PPARs are nuclear receptors, which participate in cellular lipid homeostasis and insulin action (31). Generally, PPAR α regulates the transcription of many genes involved in lipid catabolism and is highly expressed in brown fat, liver, and heart with high rates of fatty acid oxidation (32). Therefore, it is possible that dietary procyanidins as well as tea catechins promote hepatic β -oxidation of fatty acids through the activation of PPAR α . PPAR γ is the master regulator of the expression of genes that are associated with adipocyte differentiation and insulin sensitization (33). Dietary soy isoflavones, including genistein and daidzein, appear to exert antidiabetic and hypolipidemic effects through PPAR γ activation (34). Contrary to this observation, dietary conjugated linoleic acid reduced body fat mass through the downregulation of PPAR γ expression (35). As observed in our experiment, a moderate reduction of PPAR γ activity may lead to prevention of obesity induced by the consumption of the HF diet. In fact, the moderate reduction of PPAR γ /RXR activity in mice treated with their antagonist lead to a decrease in the triacylglycerol level in white adipose tissue, skeletal muscle, and liver (36). Therefore, our data suggests that a combination of the agonist-like action of PPAR α and moderate antagonist-like action of PPAR γ by metabolites from dietary polyphenols may contribute to the promotion of fatty acid β -oxidation and the reduction of lipogenic enzyme activities, thereby reducing white adipose tissue induced by the HF diet in normal rats.

5 Conclusion

We found that dietary procyanidin-rich polyphenols from AP and HP, as well as monomeric polyphenols such as TP, prevent an increase in white adipose tissue induced by the consumption of the HF diet in normal rodents, although their actions were not necessarily potent as compared with TP. The prevention of an increase in white adipose tissue mass by dietary pro-

cyanidins may be associated with the agonist-like action of PPAR α and moderate antagonist-like action of PPAR γ exerted by metabolites from dietary polyphenols. On the other hand, the prevention of an increase in white adipose tissue mass by dietary TP may be associated with both the regulation of fatty acid metabolism and the promotion of fatty acid excretion. However, the significant effects of each dietary polyphenols on enzyme activities concerning fatty acid synthesis and the levels of PPARs were not necessarily observed in this study because of data scattering. Therefore, further experiments must be performed in a large number of animals, in an obese rodent model such as Zucker fatty rat, or under normal diet conditions to evaluate more precisely the relationship between dietary procyanidins and obesity.

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