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Oligomeric Procyanidins in Apple Polyphenol Are Main Active Components for Inhibition of Pancreatic Lipase and Triglyceride Absorption

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Inhibitory effects of apple polyphenol extract (AP) and procyanidin contained in AP on in vitro pancreatic lipase activity and in vivo triglyceride absorption in mice and humans were examined. AP and procyanidin considerably inhibited in vitro pancreatic lipase activity. However, polyphenols, except for procyanidin, in AP (i.e., catechins, chalcones, and phenol carboxylic acids) showed weak inhibitory activities on pancreatic lipase. Procyanidins separated by normal-phase chromatography according to the degree of polymerization were also examined. Inhibitory effects of procyanidins increased according to the degree of polymerization from dimer to pentamer. On the other hand, pentamer or greater procyanidins showed maximal inhibitory effects on pancreatic lipase. These results suggested that with respect to in vitro pancreatic lipase inhibition, the degree of polymerization was an important factor and oligomeric procyanidin mainly contributed. Next, we performed a triglyceride tolerance test in mice and humans. Simultaneous ingestion of AP and triglyceride significantly inhibited an increase of plasma triglyceride levels in both models. These results suggested that the oligomeric procyanidins contained in AP inhibited triglyceride absorption by inhibiting pancreatic lipase activity in mice and humans.

KEYWORDS: Apple procyanidins; pancreatic lipase; hypertriglyceridemia

INTRODUCTION

It is well-known that excessive calorie intake induces obesity, which is one of the risk factors for cardiovascular disease. Obesity has become a serious public health problem in developed nations. Triglyceride is one of the energy sources that is high in calories, and suppression of triglyceride absorption is directly associated with the prevention of obesity and obesity-related diseases (*1*). Pancreatic lipase is a key enzyme for triglyceride absorption in the small intestine. This enzyme is secreted from the pancreas and hydrolyzes triglyceride into glycerol and fatty acids (*2*). Therefore, the suppression of triglyceride absorption by lipase inhibition is a major approach for preventing obesity. For example, orlistat is an agent clinically used for the management of obesity. Orlistat inhibits triglyceride absorption by the inhibition of pancreatic lipase (*3*) and its long-term administration resulted in weight loss, suggesting its efficacy for the treatment of obesity.

Apples contain several phenolic substances (i.e., chlorogenic acid, (+)-catechin, epicatechin, phloridzin, rutin, and procyanidins (condensed tannins) (*4*)). Procyanidins in apples are mainly composed of various polymerized catechins. Ohnishi-Kameyama et al. reported that condensed tannins extracted from unripe apple juice contained polymerized catechins up to pentadecamers (*5*). Kanda et al. (*6*) indicated that apple polyphenol extract (AP) also contained procyanidin in addition to monomeric polyphenol substances. Apples and AP have several biological activities such as antioxidant activity (*7*, *8*), anti-allergy activity (*6*, *9*−*11*), hair growth-promoting activity (*12*), and anti-tumor activity (*13*, *14*). In addition, some recent studies have shown that polyphenols have an efficacy for preventing obesity. Horigome et al. reported that proanthocyanidins from various plants had inhibitory effects on digestive enzymes such as trypsin, α-amylase, and lipase (*15*). Al-Mamary et al. reported that a sorghum diet containing high tannin inhibited lipase, α-amylase, and trypsin in the upper small intestine in a dose-dependent manner resulted in a decrease in the rate of weight gain and digestive efficiency in rabbits (*16*). Yoshikawa et al. reported that polyphenols extracted from *Salacia reticulata* inhibited enzymes related to fat metabolism including pancreatic lipase, lipoprotein lipase, and glycerophosphate dehydrogenase in Zucker's obese rats and that it exhibited anti-obesity effects by promoting lipid decomposition (*17*). It is suggested that AP and procyanidin contained in AP have an inhibitory effect on digestive enzymes and exert an anti-obesity effect. However, inhibitory effects of these substances on pancreatic lipase and triglyceride absorption remain unclear.

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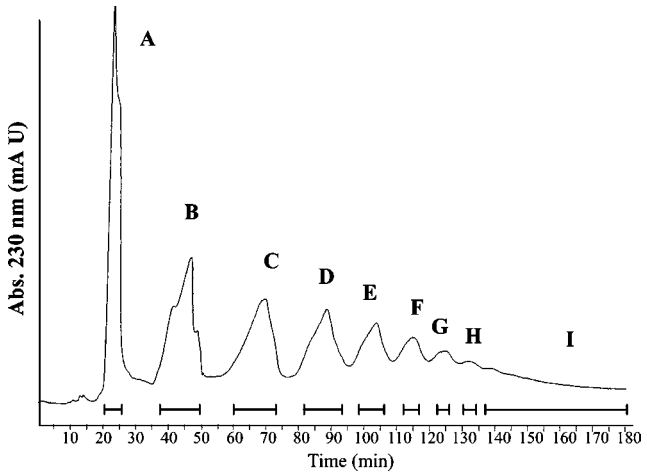

Figure 1. Profile of apple procyanidins separated by normal-phase chromatography eluted by a binary gradient with hexane–methanol–acetone. (**A**) Polyphenol monomer fraction; (**B**) procyanidin dimer fraction; (**C**) procyanidin trimer fraction; (**D**) procyanidin tetramer fraction; (**E**) procyanidin pentamer fraction; (**F**) procyanidin hexamer fraction; (**G**) procyanidin heptamer fraction; (**H**) procyanidin octamer fraction; and (**I**) procyanidin over nonamer fraction.

Table 1. Contents of Polyphenols Contained in AP[a]

	content (wt %)
Procyanidins	
dimer	13.0[b]
trimer	12.3[b]
tetramer	8.7[b]
pentamer	5.9[b]
hexamer	4.9[b]
over heptamer	20.9[b]
Flavan-3-ols	
(+)-catechin	2.0[c]
(–)-epicatechin	10.5[c]
Chalcones	
phloridzin	1.9[c]
phloretin-2′-xyloglucoside	4.6[c]
Phenolcarboxylic Acids	
chlorogenic acid	8.2[c]
p-coumaroyl quinic acid	2.6[c]
sum	95.5

[a] Each component was identified by NMR and spectral analysis (procyanidins and phloretin-2′-xyloglucoside) or by comparison of chromatograms with authentic standards ((+)-catechin, (–)-epicatechin, phloridzin, and chlorogenic acid). [b] Analyzed by RH-HPLC. [c] Analyzed by NH-HPLC.

In this study, we investigated the inhibitory effect of AP on pancreatic lipase in vitro and on triglyceride absorption in mice and humans by a single oral administration. Furthermore, procyanidin was fractionated according to the degree of polymerization by normal-phase chromatography. The inhibitory activity of each fraction was demonstrated to determine the contribution of procyanidins on lipase inhibitory activity.

MATERIALS AND METHODS

Chemicals. AP was prepared according to Yanagida et al. (*18*). Phloretin-2′-xyloglucoside and *p*-coumaroyl quinic acid were purified from AP by preparative high-performance liquid chromatography (HPLC). (+)-Catechin, (−)-epicatechin, pancreatic lipase (Type II, from porcine pancreas), and 4-methylumbelliferyl oleate (4MUO) were purchased from Sigma Chemical Co. (St. Louis, MO). Phloridzin was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Chlorogenic acid was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Preparation of Procyanidins. Procyanidins that polymerized from dimers to greater than nonamers were obtained according to Shoji et al. (*19*). Briefly, an apple procyanidin-rich fraction was prepared from AP by preparative column chromatography. The fraction obtained was dissolved in methanol and subjected to normal-phase chromatography (Inertsil PREP-SIL, GL Science, Tokyo, Japan). Procyanidins and polyphenols, except for procyanidin, were separated by a binary gradient with hexane−methanol−acetone. The eluates were collected before the procyanidin dimer was eluted, by concentrating with evaporation, and lyophilizing as an other polyphenol fractions. The eluates were obtained after the procyanidin dimers were collected, concentrated by evaporation, and lyophilized as procyanidin fractions. To investigate the relationship between the degree of polymerization and the inhibitory effects on pancreatic lipase activity, we further fractionated procyanidin contained in AP according to the degree of polymerization. Each procyanidin oligomer fraction was collected by using the same apparatus described earlier (**Figure 1**). Each procyanidin oligomer fraction was concentrated by a rotary evaporator and lyophilized. The contents of each fraction are shown in **Table 1.**

Measurement of Pancreatic Lipase Activity. Pancreatic lipase activity was measured using 4MUO as the substrate (*20*). Twent-five microliters of the sample solution dissolved in water and 25 μL of the pancreatic lipase solution (1 mg/mL) were mixed in the well of a microtiter plate. Fifty microliters of 4MUO solution (0.1 mM) dissolved in Dulbecco's phosphate buffered saline was then added to initiate the enzyme reaction. After incubation at 23 °C for 20 min, 100 μL of 0.1 M sodium citrate (pH 4.2) was added to stop the reaction. The amount of 4-methylumbelliferone released by lipase was measured using a fluorescence microplate reader (Spectramax Gemini, Molecular Devices Co. Ltd., Sunnyvale, CA) at an excitation wavelength of 320 nm and an emission wavelength of 450 nm. The inhibitory activity was expressed as a percentage of the control. The 50% inhibition concentration (IC_{50}) of the test sample was obtained from the least-squares regression line of the plots of the logarithm of the sample concentration (log) versus the inhibitory activity (%). These measurements were performed in triplicate and expressed as mean ± standard error (SEM).

Oral Triglyceride Tolerance Test in Mice. Male ddY mice aged 9 weeks were housed four in each cage in an air-conditioned room (22 ± 2 °C) with a 12 h light cycle (0600−1800). The animals were maintained according to the Guidelines for Animal Experimentation of Asahi Breweries Ltd., compiled from the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. Before the administration of the test sample, the mice were starved for 20 h. For the triglyceride tolerance test, the sample was orally administered to the mice at the indicated concentration followed by 10 mL/kg body weight (BW) of corn oil. An equal volume of distilled water and corn oil was administered to the control mice. The mice were analyzed for chronological changes in plasma triglyceride with eight animals studied at each time point. Blood samples were taken from the intraorbital vein using a glass capillary containing sodium heparin and centrifuged at 2000*g* for 5 min to obtain plasma as a sample. Triglyceride in plasma was measured using Triglyceride E-test Wako (Wako Pure Chemical Industries Ltd.) according to the manufacturer's instructions. Data were analyzed using analysis of variance (ANOVA). The multiple comparisons were made using Tukey's honestly significantly different (HSD) test.

Human Study. The human study was approved by the Ethics Review Board in accordance with the Helsinki Declaration. The subjects were six healthy male volunteers with a plasma triglyceride level of 1.50 g/L or less at a health checkup prior to this study. The fat content of a high-fat diet was established as 40 g. We conducted a double-blind cross study involving a 2 week period of recovery. A diet containing 40 g of triglyceride with 10 control capsules or 10 AP containing capsules (600 or 1500 mg, respectively) was given to the subjects. The subjects fasted for 9 h from 12:00 a.m. of the study day, and the study began at 9:00 a.m. During the study period, the subjects fasted and were allowed to ingest only a small amount of water or caffeine-free tea. Exercise was restricted to resting in the sitting position or light

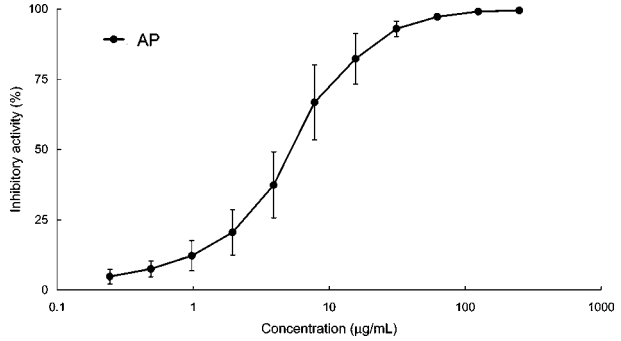

Figure 2. Inhibitory effects of AP on pancreatic lipase. Values are mean ± SEM.

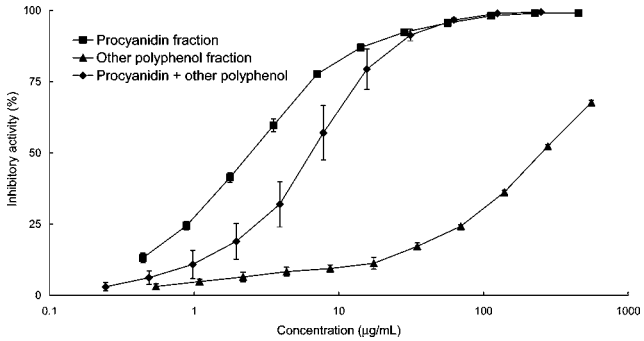

Figure 3. Inhibitory effects of the procyanidin fraction and the other polyphenol fraction in AP on pancreatic lipase activity. Values are mean ± SEM. Samples were prepared by normal-phase chromatography according to Shoji et al. (*19*).

work. Blood was collected through the radial vein before the ingestion of the test diet and at 1, 2, 3, 4, and 6 h after the ingestion. Blood samples were analyzed at Mitsubishi Chemical BCL Co. Ltd. (Tokyo, Japan). An inquiry and questionnaire survey were conducted. The fat containing (40 g) diet was prepared by mixing 200 g of a commercially available corn cream of potato soup with 17 g of butter and 14 g of melted lard. The significance of differences in the triglyceride concentration between the control and AP groups was tested using the paired *t*-test.

RESULTS

Inhibitory Effects of AP on Pancreatic Lipase Activity in Vitro. The inhibitory activity of AP against pancreatic lipase is shown in **Figure 2**. AP inhibited pancreatic lipase activity in a dose-dependent manner. Treatment with AP at a concentration of 250 μg/mL almost completely inhibited the activity of pancreatic lipase. The IC_{50} value was 5.6 μg/mL (**Table 2**).

Inhibitory Effects of Procyanidin Fraction and Other Polyphenol Fractions on Pancreatic Lipase Activity in Vitro. The procyanidin fractions and the other polyphenol fractions, both of which were extracted from AP, inhibited pancreatic lipase activity in a dose-dependent manner (**Figure 3**). The IC_{50} value of the procyanidin fraction was 1.4 μg/mL (**Table 2**). The inhibitory activity of the procyanidin fraction was more potent than that of AP. On the contrary, the other polyphenol fractions showed weak inhibitory activity on pancreatic lipase (IC_{50}; 115.9 μg/mL). To investigate the contribution of these two fractions to the inhibitory activity of AP on pancreatic lipase, we mixed the procyanidin fraction and the other polyphenol fraction according to the ratio of their components (procyanidin + other polyphenol fraction) and measured the inhibitory activity of the mixed fraction. The mixed fraction also inhibited the pancreatic lipase activity in a dose-dependent manner. The IC_{50} value of this fraction was 6.7 μg/mL, which was almost the same as that of AP.

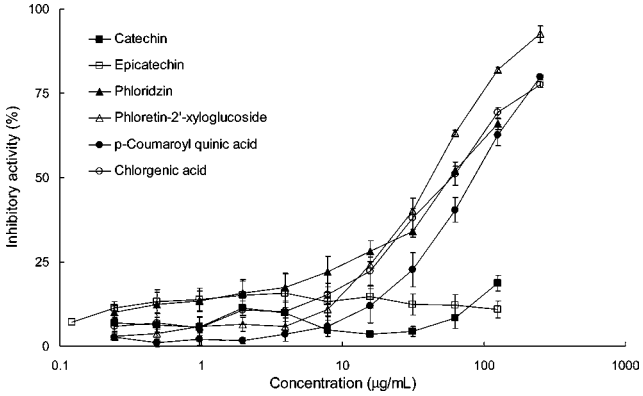

Figure 4. Inhibitory effects of polyphenols except for procyanidins on pancreatic lipase. Values are mean ± SEM.

Table 2. Inhibitory Effects of AP and Polyphenols Contained in AP on Pancreatic Lipase

	IC_{50} (μg/mL)
AP	5.6
procyanidin fraction	1.4
other polyphenol fractions	115.9
procyanidin + other polyphenol fractions	6.7
Procyanidins	
dimer	>125
trimer	32.9
tetramer	6.7
pentamer	1.3
hexamer	2.3
heptamer	0.7
octamer	1.9
over nonamer	0.9
Flavan-3-ols	
(+)-catechin	>125
(−)-epicatechin	>125
Chalcones	
phloridzin	58.7
phloretin-2′-xyloglucoside	44.6
Phenolcarboxylic Acids	
chlorogenic acid	59.8
p-coumaroyl quinic acid	89.0

Inhibitory Effects of Polyphenols in AP Except for Procyanidin on Pancreatic Lipase Activity in Vitro. To evaluate the inhibitory activity of the other polyphenol fraction on pancreatic lipase in detail, the inhibitory activity of each of the main components contained in the other polyphenol fraction was also examined (**Figure 4**). Standard products ((+)-catechin, (−)-epicatechin, phloridzin, and chlorogenic acid) and products purified from AP (phloretin-2′-xyloglucoside and *p*-coumaroyl quinic acid) were used. As neither (+)-catechin nor (−)-epicatechin showed inhibitory activity on pancreatic lipase, their IC_{50} values could not be calculated. IC_{50} values of phloridzin, phloretin-2′-xyloglucoside, chlorogenic acid, and *p*-coumaroyl quinic acid were higher than that of AP (IC_{50}; 58.7, 44.6, 59.8, and 89.0 μg/mL, respectively; **Table 2**). All polyphenols contained in the other polyphenol fraction showed weak inhibitory activity on pancreatic lipase.

Inhibitory Effects of Procyanidins Isolated from AP with Respect to the Degree of Polymerization on Pancreatic Lipase Activity in Vitro. Procyanidins contained in AP were

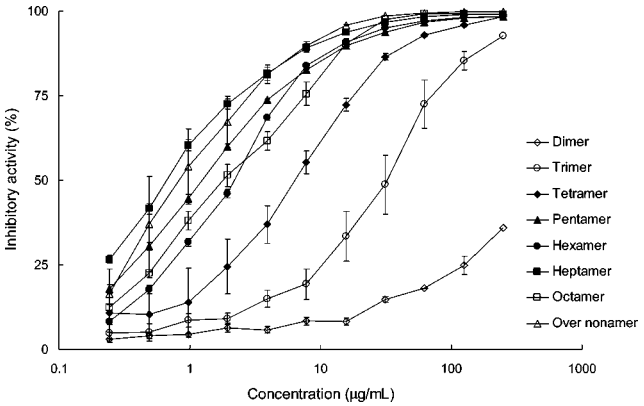

Figure 5. Inhibitory effects of procyanidins on pancreatic lipase activity. Procyanidins were fractionated according to the degree of polymerization. Each fraction was separated by normal-phase chromatography according to Shoji et al. (*19*). Values are mean ± SEM.

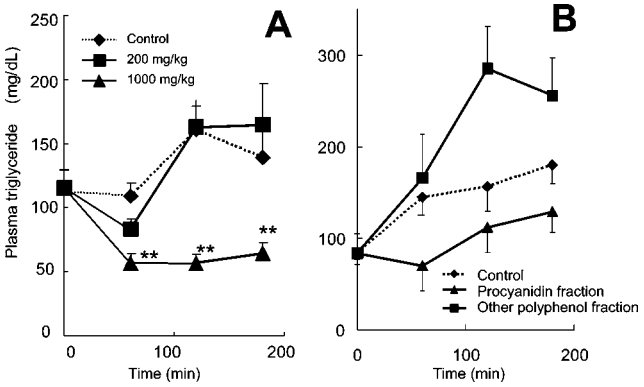

Figure 6. Inhibitory effects of a single oral administration of AP on plasma triglyceride levels in corn oil loaded mice (**A**). Effects of the procyanidin fractions and other polyphenol fractions on triglyceride absorption in mice (**B**). Values are mean ± SEM. *Significantly different from control, $P < 0.05$. **Significantly different from control, $P < 0.01$.

fractionated by normal-phase chromatography according to the degree of polymerization. The inhibitory activity of individual fractions on in vitro pancreatic lipase activity was measured. For measurement, fractions consisting of dimers to octamers and those consisting of nonamers or greater were employed (**Figure 5**). All fractions inhibited pancreatic lipase activity in a dose-dependent manner. Inhibitory activities of procyanidins consisting of dimers to pentamers increased with an increase in the degree of polymerization (IC_{50}; >125, 32.9, 6.7, and 1.3 μg/mL; **Table 2**). However, the inhibitory activity of procyanidin consisting of a pentamer or greater seemed to have reached the maximal level. The IC_{50} values of these procyanidins ranged from 0.7 to 2.3 μg/mL.

Inhibitory Effects of AP on Triglyceride Absorption in Mice. The influence of AP on triglyceride absorption in mice is shown in **Figure 6A**. In the control group, plasma triglyceride levels increased after ingestion of corn oil. Administration of AP at 200 mg/kg decreased the plasma triglyceride at 60 min after corn oil loading as compared with the start time. In the group receiving 1000 mg/kg, there was no increase in the plasma triglyceride level as compared to the start time during the test period. Administration of AP at 1000 mg/kg significantly decreased the plasma triglyceride at 60, 120, and 180 min after administration as compared with the control group ($P < 0.01$). The effects of the procyanidin fractions and the other polyphenol fractions on triglyceride absorption in mice were also investigated (**Figure 6B**). The amount of each fraction contained in 200 mg/kg AP was used. The procyanidin fraction tended to inhibit triglyceride absorption as compared with the control group. However, the other polyphenol fraction showed no effect on triglyceride absorption. The area under the curve was significantly reduced in the procyanidin fraction group as compared to the other polyphenol fraction group ($P < 0.05$, data not shown).

Table 3. Effect of Apple Polyphenol Extract on Triglyceride Absorption in Humans[a]

	0 h	1 h	2 h	3 h	4 h	6 h
AP (600 mg)	75 ± 4	99 ± 5	110 ± 6	125 ± 15	130 ± 16	77 ± 7[b]
AP (1500 mg)	86 ± 13	102 ± 16	103 ± 20	114 ± 26	119 ± 23	108 ± 18

[a] Values are mean ± SEM. [b] Significantly different from placebo, $P < 0.05$.

Inhibitory Effects of AP on Triglyceride Absorption in Humans. The subjects were six patients with fasting serum triglyceride levels ranging from 40 to 147 mg/dL, with a mean age of 35.5 ± 1.2 years (range, 30−43 years). **Table 3** shows the changes in the serum triglyceride level after a test diet containing AP or control AP-free capsules were given. The serum triglyceride level reached a maximum at 4 h after 40 g of triglyceride administration. As shown in **Table 3**, the test diet containing 600 mg of AP significantly inhibited triglyceride elevation at 6 h after ingestion, indicating its inhibitory effects on triglyceride absorption. Ingestion of AP did not cause any side effects such as diarrhea or constipation.

DISCUSSION

In this study, the inhibitory effects on pancreatic lipase of AP and the procyanidin contained in it were investigated. In addition, the influence of these substances on triglyceride absorption in mice and humans was examined. AP inhibited in vitro pancreatic lipase activity in a dose-dependent manner. To examine the contribution of procyanidin to the inhibitory effects of AP on pancreatic lipase, we conducted a study using fractions isolated from AP. The pancreatic lipase inhibitory activities of two polyphenol fractions were measured. These fractions contained the procyanidins and polyphenols except for procyanidin (called procyanidin fractions and other polyphenol fractions, respectively) found in AP. The procyanidin fraction had a more potent inhibitory effect on pancreatic lipase as compared with AP. The activities of the other polyphenol fractions were markedly reduced. The mixture of these two fractions in accordance with their proportions in AP could reproduce the inhibitory activity of AP. These results clearly indicated that procyanidins were the major contributor to the inhibitory effect of AP on pancreatic lipase. Kanda et al. (*6*) investigated the inhibitory effect of AP on hyaluronidase. AP inhibited hyaluronidase activity in proportion to the procyanidin content, suggesting that procyanidins interact with proteins other than pancreatic lipase. Polyphenols other than procyanidins contained in AP are mainly classified into three types: (i) flavan-3-ols, (ii) chalcones, and (iii) phenolcarboxylic acids. Inhibitory activities of these polyphenols were less marked than that of AP. In particular, inhibitory activities of (+)-catechin and (−)-epicatechin were weak. Phenolcarboxylic acids and chalcones showed similar inhibitory effects. However, all of the inhibitory activities of these polyphenols were less than that of AP. These results suggested the small contribution of these polyphenols to pancreatic lipase inhibition. Procyanidins in apples are composed of dimers to pentadecamers (*5*). In this study, we fractionated procyanidins according to the degree of polymer-

ization, which ranged from dimers to over nonamers, and investigated the relationship between degree of polymerization and inhibitory activity on pancreatic lipase. In the case of procyanidins between dimers and pentamers, the increase in the degree of polymerization of procyanidin was accompanied by an increase in inhibitory activities on pancreatic lipase. In addition, when procyanidins consisting of a pentamer or greater were examined, inhibitory activities of these procyanidins seemed to reach a maximal level. These results suggested that the degree of polymerization was an important factor in determining the inhibitory activity of procyanidin on pancreatic lipase as well as in indicating the importance of the degree of polymerization in the interaction between procyanidin and proteins including pancreatic lipase. Generally, it is assumed that tannins including procyanidins bind to proteins (*21*). Tannins were thought to exert their inhibitory effect on enzymes by binding to proteins. The tannin−protein bond is considered to be nonspecific. According to this idea, the inhibitory activity of procyanidin on enzymes seemed to be independent of the degree of polymerization. However, our results showed a clear relationship between the degree of polymerization and their inhibitory activities on pancreatic lipase. Several studies have been undertaken to elucidate the relationship between the degree of polymerization in polyphenols and the enzyme inhibition. Shoji et al. (*22*) investigated inhibitory activities on tyrosinase by procyanidins consisting of a pentamer or smaller, which were prepared from AP. It was reported that there was no relationship between the degree of polymerization and enzyme inhibition. Inhibitory effects of condensed tannins and procyanidins on an angiotensin converting enzyme were also reported. The activities of condensed tannins increased with the degree of polymerization from monomer to trimer (*23*). Among procyanidins consisting of dimers, tetramers, or hexamers, the tetramer procyanidin showed the most potent activity (*24*). In the case of pancreatic lipase, at least pentameric procyanidin was needed for sufficient inhibition. The contents of tetramer or greater procyanidins in AP were approximately 40% (**Table 1**). Considering the composition and inhibitory activities, it was suggested that these oligomeric procyanidins were the main contributors to the inhibitory effect of AP on pancreatic lipase. It was reported that hydrophobic effects, hydrogen bonds, and proline residues influenced the tannin−protein binding (*25*). However, we could not conclude which factor determined the relationship between pancreatic lipase inhibitory activities and degree of polymerization. Recently, the development of analytical methods revealed the chemical structure of polyphenols, for example, the three-dimensional structures of procyanidins (*26*) and the mode of the binding of procyanidin B3 to saliva protein (*27*). In the future, the three-dimensional structures of oligomeric procyanidins and pancreatic lipase binding should be further investigated.

AP and fractionated procyanidin inhibited the postprandial elevation of plasma triglyceride level in mice. On the other hand, the other polyphenol fraction showed no effects on plasma triglyceride. Some polyphenols have been reported to inhibit pancreatic lipase and triglyceride absorption (*20*). These studies indicated gallocatechin gallate, catechin gallate (*28*), epigallocatechin gallate, and epicatechin gallate (*29*) as the main active ingredients. However, AP does not contain these polyphenols that have the galloyl moiety (**Table 1**). In our study, procyanidins, especially oligomeric procyanidins in AP, seemed to be the main active ingredient for the inhibitory effect on triglyceride absorption. The efficacy of AP on triglyceride absorption in humans was also demonstrated. Procyanidins are thought to act as an active ingredient. To our knowledge, this is the first study that has reported that procyanidins can prevent postprandial hypertriglyceridemia. If the mechanism is based on the inhibitory effects on lipase activity, it is expected that the intake of AP causes an increase of gas in the bowel resulting in a bloating sensation, abdominal wind, and diarrhea. However, the increase of frequency in abdominal symptoms was not observed in this study and a number of other animal and human studies (*30*−*33*). Moreover, it is reported that the total dietary intake of polyphenols should be approximately 1 g/day (*34*). The effective dose of AP for the lipase inhibition was lower than the dairy intake of polyphenols. These results suggested the safety of AP for preventing postprandial hypertriglyceridemia.

It has been demonstrated that the plasma concentration of triglyceride in a postprandial state is an independent predictor of cardiovascular disease (*35*). AP may prevent obesity and cardiovascular disease through the inhibition of lipase and postprandial hypertriglyceridemia. However, no data on the relationship between the ingestion of AP and cardiovascular disease have been published, and this issue should be further investigated in the future.

In conclusion, we found that AP inhibited pancreatic lipase and prevented postprandial elevation of the plasma triglyceride level. In addition, oligomeric procyanidins were highly effective in pancreatic lipase inhibition. Therefore, it was suggested that oligomeric procyanidin was the main contributor to the effect of AP on inhibiting triglyceride absorption. AP may relieve obesity via a lipase inhibiting activity and may be effective for obesity-related diseases.

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LITERATURE CITED

(1) Carek, P. J.; Dickerson, L. M. Current concepts in the pharmacological management of obesity. *Drugs* **1999**, *57*, 883−904.
(2) Lowe, M. E. Pancreatic triglyceride lipase and colipase: Insights into dietary fat digestion. *Gastroenterology* **1994**, *107*, 1524−1536.
(3) Hvizdos, K. M.; Markham, A. Orlistat: A review of its use in the management of obesity. *Drugs* **1999**, *58*, 743−760.
(4) Nonaka, G.; Hsu, F.; Nishioka, I. Structures of dimeric, trimeric, and tetrameric procyanidins from *Areca catechu L. J. Chem. Soc., Chem. Commun.* **1981**, 781−784.
(5) Ohnishi-Kameyama, M.; Nagata, T.; Yanagida, A.; Kanda, T. Identification of catechin oligomers from apple (*Malus pumila* cv. Fuji) in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and fast-atom bombardment mass spectrometry. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 31−36.
(6) Kanda, T.; Yanagida, A.; Tanabe, M.; Akiyama, H.; Goda, Y.; Toyoda, M.; Teshima, R.; Saito, Y. Inhibitory effects of apple polyphenol on induced histamine release from RBL-2H3 cells and rat mast cells. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1284−1289.
(7) Leontowicz, H.; Leontowicz, M.; Gorinstein, S.; Lojek, A.; Ciz, M.; Soliva-Fortuny, R.; Martin-Belloso, O.; Jung, S.-T.; Trakhtenberg, S. Comparative content of some bioactive compounds in apples, peaches, and pears and their influence on lipids and antioxidant capacity in rats. *J. Nutr. Biochem.* **2002**, *13*, 603−610.
(8) Schaefer, S.; Baum, M.; Eisenbrand, G.; Janzowski, C. Modulation of oxidative cell damage by reconstituted mixtures of phenolic apple juice extracts in human colon cell lines. *Mol. Nutr. Food Res.* **2006**, *50*, 413−417.

(9) Akiyama, H.; Sakushima, J.; Teshima, R.; Toyoda, M.; Taniuchi, S.; Kojima, T.; Kobayashi, Y.; Kanda, T.; Yanagida, A. Antiallergic effect of apple polyphenols on the allergic model mouse. *Biol. Pharm. Bull.* **2000**, *23*, 1370−1373.

(10) Akiyama, H.; Sato, Y.; Watanabe, T.; Nagaoka, M. H.; Yoshioka, Y.; Teshima, R.; Sawada, J.; Goda, Y.; Maitani, T.; Shoji, T.; Kanda, T.; Yamada, K.; Totsuka, M. Dietary unripe apple polyphenol inhibits the development of food allergies in murine models. *FEBS Lett.* **2005**, *579*, 4485−4491.

(11) Kishi, K.; Saito, M.; Saito, T.; Kumemura, M.; Okamatsu, H.; Okita, M.; Takazawa, K. Clinical efficacy of apple polyphenol for treating cedar pollinosis. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 829−832.

(12) Takahashi, T.; Kamimura, A.; Kagoura, M.; Toyoda, M.; Morohashi, M. Investigation of the topical application of procyanidin oligomers from apples to identify their potential use as a hair-growing agent. *J. Cosmet. Dermatol.* **2005**, *4*, 245−249.

(13) Lapidot, T.; Kanner, J.; Walker, M. D. Can apple antioxidants inhibit tumor cell proliferation? Generation of H_2O_2 during interaction of phenolic compounds with cell culture media. *J. Agric. Food Chem.* **2002**, *50*, 3156−3160.

(14) Gossé, F.; Guyot, S.; Roussi, S.; Lobstein, A.; Fischer, B.; Seiler, N.; Raul, F. Chemopreventive properties of apple procyanidins on human colon cancer-derived metastatic SW620 cells and in a rat model of colon carcinogenesis. *Carcinogenesis* **2005**, *26*, 1291−1295.

(15) Horigome, T.; Kumar, R.; Okamoto, K. Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes in vitro and in the intestine of rats. *Br. J. Nutr.* **1988**, *60*, 275−285.

(16) Al-Mamary, M.; Al-Aghbari, A.; Al-Habori, M.; Al-Obeidi, A. In vivo effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. *Nutr. Res.* **2001**, *21*, 1393−1401.

(17) Yoshikawa, M.; Shimoda, H.; Nishida, N.; Takada, M.; Matsuda, H. *Salacia reticulata* and its polyphenolic constituents with lipase inhibitory and lipolytic activities have mild antiobesity effects in rats. *J. Nutr.* **2002**, *132*, 1819−1824.

(18) Yanagida, A.; Kanda, T.; Shoji, T.; Ohnishi-Kameyama, M.; Nagata, T. Fractionation of apple procyanidins by size-exclusion chromatography. *J. Chromatogr., A* **1999**, *855*, 181−190.

(19) Shoji, T.; Masumoto, S.; Moriichi, N.; Kanda, T.; Ohtake, Y. Apple (*Malus pumila*) procyanidins fractionated according to the degree of polymerization using normal-phase chromatography and characterized by HPLC-ESI/MS and MALDI-TOF/MS. *J. Chromatogr., A* **2006**, *1102*, 206−213.

(20) Kurihara, H.; Asami, S.; Shibata, H.; Fukami, H.; Tanaka, T. Hypolipemic effect of *Cyclocarya paliurus* (Batal) Iljinskaja in lipid-loaded mice. *Biol. Pharm. Bull.* **2003**, *26*, 383−385.

(21) Hagerman, A. E.; Butler, L. G. The specificity of proanthocyanidin−protein interactions. *J. Biol. Chem.* **1981**, *256*, 4494−4497.

(22) Shoji, T.; Masumoto, S.; Moriichi, N.; Kanda, T.; Kobori, M.; Shinmoto, H.; Tsushida, T. Procyanidin trimers to pentamers fractionated from apple inhibit melanogenesis in B16 mouse melanoma cells. *J. Agric. Food Chem.* **2005**, *53*, 6105−6111.

(23) Uchida, S.; Ikari, N.; Ohta, H.; Niwa, M.; Nonaka, G.; Nishioka, I.; Ozaki, M. Inhibitory effects of condensed tannins on angiotensin converting enzyme. *Jpn. J. Pharmacol.* **1987**, *43*, 242−246.

(24) Ottaviani, J. I.; Actis-Goretta, L.; Villordo, J. J.; Fraga, C. G. Procyanidin structure defines the extent and specificity of angiotensin I converting enzyme inhibition. *Biochimie* **2006**, *88*, 359−365.

(25) Haslam, E. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *J. Nat. Prod.* **1996**, *59*, 205−215.

(26) Muranaka, A.; Yoshida, K.; Shoji, T.; Moriichi, N.; Masumoto, S.; Kanda, T.; Ohtake, Y.; Kobayashi, N. Chiral recognition of apple procyanidins by complexation with oxotitanium phthalocyanine. *Org. Lett.* **2006**, *8*, 2447−2450.

(27) Simon, C.; Barathieu, K.; Laguerre, M.; Schmitter, J. M.; Fouquet, E.; Pianet, I.; Dufourc, E. J. Three-dimensional structure and dynamics of wine tannin−saliva protein complexes. A multitechnique approach. *Biochemistry* **2003**, *42*, 10385−10395.

(28) Ikeda, I.; Tsuda, K.; Suzuki, Y.; Kobayashi, M.; Unno, T.; Tomoyori, H.; Goto, H.; Kawata, Y.; Imaizumi, K.; Nozawa, A.; Kakuda, T. Tea catechins with a galloyl moiety suppress postprandial hypertriacylglycerolemia by delaying lymphatic transport of dietary fat in rats. *J. Nutr.* **2005**, *135*, 155−159.

(29) Kurihara, H.; Shibata, H.; Fukui, Y.; Kiso, Y.; Xu, J. K.; Yao, X. S.; Fukami, H. Evaluation of the hypolipemic property of *Camellia sinensis* Var. *ptilophylla* on postprandial hypertriglyceridemia. *J. Agric. Food Chem.* **2006**, *54*, 4977−4981.

(30) Shoji, T.; Akazome, Y.; Kanda, T.; Ikeda, M. The toxicology and safety of apple polyphenol extract. *Food Chem. Toxicol.* **2004**, *42*, 959−967.

(31) Akazome, Y.; Kanda, T.; Ohtake, Y.; Hashimoto, H.; Kametani, N.; Sato, K.; Nakamura, T.; Kajimoto, Y. Evaluation of safety of excessive intake and efficacy of long-term intake of beverage containing polyphenols derived from apples. *Jpn. Pharmacol. Ther.* **2005**, *33*, 893−911.

(32) Nagasako-Akazome, Y.; Kanda, T.; Ikeda, M.; Shimasaki, H. Serum cholesterol-lowering effect of apple polyphenols in healthy subjects. *J. Oleo Sci.* **2005**, *54*, 143−151.

(33) Enomoto, T.; Nagasako-Akazome, Y.; Kanda, T.; Ikeda, M.; Dake, Y. Clinical effects of apple polyphenols on persistent allergic rhinitis: A randomized double-blind placebo-controlled parallel arm study. *J. Invest. Allergol. Clin. Immunol.* **2006**, *16*, 283−289.

(34) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **2000**, *130*, 2073−2085.

(35) Cohn, J. S. Postprandial lipemia: Emerging evidence for atherogenicity of remnant lipoproteins. *Can. J. Cardiol.* **1998**, *14* (Suppl. B), 18−27.

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