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Effects of Polyphenols from Water Chestnut (Trapa japonica) on Lipid Metabolism

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Abstract

Water chestnut (*Trapa japonica*), a uniquely shaped floating plant with thorns, contains many peculiar polyphenols. These polyphenols have been shown to exhibit high antioxidant activity and suppress postprandial blood glucose level rise. In recent years, obesity and lipid metabolism disorders have become a worldwide problem. Therefore, with the aim of investigating the effects of water chestnut polyphenols on lipid metabolism, we conducted in vitro studies, animal experiments, and human clinical studies (randomized, double-blind, placebo-controlled, and parallel-group comparative studies). Water chestnut polyphenol exhibited lipase inhibitory activity and inhibited fat accumulation in 3T3-L1 cells. In contrast, animal experiments using mice revealed that triglycerides and free fatty acids in the blood plasma of the polyphenol-treated group were significantly lower than those in the control group. Human clinical studies showed that the consumption of water chestnut tea significantly reduced visceral fat area and abdominal circumference; however, no significant difference was observed from the results for the placebo group. These results suggest a possible preventive effect of water chestnut polyphenols on the development of metabolic syndrome. This study was registered with the UMIN Clinical Trial Registry (UMIN000038165, October 4, 2019).

Introduction

In 1999, the World Health Organization first proposed the concept of metabolic syndrome, which is a combination of risk factors for atherosclerosis organized in terms of insulin resistance, along with its diagnostic criteria [1]. Today, the concept and diagnostic criteria of metabolic syndrome differ among countries and institutions, but there are two major schools of thought: one based on overlapping risk factors [2, 3] and the other based on insulin resistance and visceral fat [4, 5]. While the former is the mainstream approach worldwide, Japan has adopted the visceral-fat-based approach and diagnostic criteria [5].

The Global Burden of Disease report revealed that in 2015, 1.77 million children and 6.37 million adults worldwide were obese and that the number of health problems caused by obesity doubled compared to that in 1980 [6]. According to the Japan National Health and Nutrition Survey conducted in 2019, the proportion of those strongly suspected of having metabolic syndrome was 29.8% for men and 9.5% for women aged 40–74 years, and the proportion of those considered to be in the preliminary group was 24.7% for men and 7.2% for women [7]. The combined proportion of those strongly suspected of having metabolic syndrome and those in the preliminary group is 1 in 2 men and 1 in 6 women.

One of the diagnostic criteria for metabolic syndrome in Japan is waist circumference (abdominal circumference), with measurements of ≥ 85 cm for men and ≥ 90 cm for women as a prerequisite [5]. These values correspond to a visceral fat area of ≥ 100 cm^2 on an abdominal CT scan. Therefore, reducing visceral fat area is expected to lead to the prevention or elimination of metabolic syndrome. Causes of visceral fat accumulation include lack of exercise and disordered eating habits, which are difficult to correct in the busy lifestyles of modern society.

To promote preventive measures against obesity, research has been vigorously conducted on functional foods that can be easily and efficiently ingested. For example, the anti-obesity effects of catechins in green tea have been reported in mice [8], rats [9], and humans [10].

Water chestnut (*Trapa japonica*) is a uniquely shaped floating plant with thorns that grows naturally in ponds, marshes, and creeks. It is a rare plant that spreads its leaves on the surface of water and produces spiny fruits. We previously isolated and purified polyphenols contained in water chestnut berries and determined their structural formulas, identifying them as eugeniin, 1,2,3,6-tetra-*O*-galloyl-β-D-glucopyranose (TGG), and trapain, which are hydrolyzed polyphenols with multiple gallic acids [11]. Trapain, which is named after the scientific name of water chestnut, is a rare polyphenol that was previously believed to be found only in castor beans [12]. We have previously investigated the functionalities of water chestnut polyphenols (WCPs) and revealed their high antioxidant activities and inhibition of postprandial blood glucose elevation [11, 13]. However, their functionality against metabolic syndrome has not been reported to date.

In this study, we first investigated the lipase inhibitory activity of polyphenols contained in the hull of rinderpests and the inhibition of fat accumulation by 3T3-L1 progenitor adipocytes in mice as in vitro experiments. Then, as in vivo experiments, we fed mice with a high-fat diet comprising castor bean polyphenols and investigated their effects on body fat and blood triglycerides. In addition, we conducted a human clinical study in which study participants with a high BMI (kg/m^2), visceral fat area of ≥ 80 cm^2, and/or abdominal circumference of ≥ 81 cm consumed the powdered husk of water chestnuts in the form of tea for 8 weeks to verify its effectiveness in alleviating metabolic syndrome.

Materials and Methods

Sample Preparation

Water chestnut plants growing wild in a creek in Kanzaki City, Saga Prefecture, Japan were collected and dried, and the fruits (starchy parts) were removed and crushed. Then, 2 L of 80% ethanol was added to 100 g of this crushed material in a triangular flask (2 L); the material was soaked for one day and night and subsequently filtered through filter paper (No. 1, ADVANTEC, Japan), following which the ethanol was removed with an evaporator. Further filtration was carried out and the mixture was concentrated in an evaporator. The concentrated solution was poured through a column (φ80 × 700 mm, DIAON HP20, Mitsubishi Chemical Co., Ltd., Japan), distilled water was poured through to remove impurities, 80% ethanol was poured through, and the resulting solution was concentrated and dried in an evaporator to make crude WCP.

For the pancreatic lipase activity test and the experiment using 3T3 cells, WCP was dissolved in ethanol and diluted with a buffer solution or medium to achieve an ethanol concentration of $\leq 1\%$ for each test. Water chestnut tea (WCT) used in the human clinical studies was made by crushing the dried husk of the water chestnut, roasting it, and then powdering it.

Reagents

For high-performance liquid chromatography (HPLC), HPLC grade solvents were purchased from FUJIFILM Wako Pure Chemicals Co. (Japan). Other reagents, unless otherwise noted, were special grade manufactured by FUJIFILM Wako Pure Chemical Co. (Japan).

Analytical Methods for Polyphenols

The three polyphenols (eugeniin, TGG, and trapaenin) in the tea used in the human clinical studies were analyzed using a previously reported HPLC method [11]. In addition, based on a previous study by Sun et al., the total polyphenol content in the mustard tea was determined using the Folin–Ciocâlteu method [14]. In brief, 100 µL of the sample solution and 100 µL of Folin–Ciocâlteu reagent diluted two-fold with water were added to a microtube, stirred, and allowed to stand for 3 min at room temperature (25°C). Then, 100 µL of 10% sodium carbonate solution was added, stirred, and allowed to react for 60 min at room temperature under darkness, following which the solution was centrifuged (3000 rpm, 15 min). Then, 200 µL of the supernatant was placed in a microplate, and the absorbance was measured at 750 nm using a microplate reader (Synergy HT, Bio-Tek Instruments Inc., USA). Total polyphenol content was expressed as equivalent to gallic acid per 100 g.

Evaluation Method for Lipase Inhibitory Activity

Lipase inhibitory activity was investigated based on the principle of enzyme assay method using S-acyl compounds, which are synthetic substrates that are highly reactive with the pancreatic lipase, in combination with reagents for measuring SH groups. In microtubes, 50 µL of each sample solution were added, to which 15 µL of porcine pancreatic lipase (0.05 mg/mL; Type II, Sigma-Aldrich) which were dissolved in 0.1 M Tris-HCl buffer solution (pH 7.5) were added. In addition, 200 µL of the chromogenic solution (0.1 mg/mL 5,5'-dithiobis(2-nitrobenzoic acid)) from Lipase Kit S (Dainippon Sumitomo Pharma Co., Ltd., Japan) was added. After 5 min of pre-incubation at 30°C, 20 µL of the Lipase Kit S substrate solution (6.69 mg/mL dimercaprol tributyrate and 5.73 mg/mL sodium dodecyl sulfate) was added. Incubation was carried out at 30°C for 30 min under darkness. Then, 400 µL of the reaction stopper from Lipase Kit S was added. The absorbance at 412 nm was measured using a microplate reader (Synergy HT, BioTek Instruments Inc., USA). The same experiment was performed using water instead of the sample solution as a control and (−)-epigallocatechin gallate (EGCG, ≥ 98%, Nagara Science Inc.), a type of green tea polyphenol, as a comparison of WCPs. The blank was made by adding the substrate solution after adding the reaction stopper solution. The lipase inhibitory activity was calculated by the following equation:

$$\text{Lipase inhibitory activity} = 100 - \{(S-SB)/(C-CB)\} \times 100 \quad (1)$$

where S and SB represent the absorbance of samples and sample blanks, respectively, while C and CB represent the absorbance of controls and control blanks, respectively.

Fat Accumulation Study in 3T3-L1 Cells [15–17]

Mouse adipose progenitor cells, 3T3-L1 cells (Human Science Foundation, Japan), were seeded in 24-well plates at a concentration of 1×10^5 cells/well and cultured in a 5% CO_2 incubator (37°C) for 3 days. Then, the cells were differentiated into adipocytes using differentiation-inducing reagents (3-isobutyl-1-methylxanthine, dexamethasone, and insulin) from the Adipogenesis Assay Kit (Cayman Chemical Company, USA). The medium used was Dulbecco's Modified Eagle's Medium (DMEM; Nissui Pharmaceutical Co., Ltd., Japan) supplemented with 1% Penicillin-Streptomycin and either 10% fetal calf serum (FCS) during cellular succession or 10% fetal bovine serum (FBS) at cell differentiation. To the medium, 25, 50, and 100 μg/mL WCP solutions were added, resulting in final concentrations of 12.5, 25, and 50 μg/mL, respectively. For comparison, 50 μg/mL of EGCG was added to the medium to achieve a final concentration of 25 μg/mL). After 3 days of incubation, the medium was replaced with a freshly prepared sample solution-added medium. The same experiment was also performed on undifferentiated progenitor cells using the Adipogenesis Assay Kit. The cells were divided into three groups for the experiment: only the progenitor cells (undifferentiated), cells to which only medium was added as untreated control, and cells to which berberine chloride was added at a final concentration of 1 μg/mL as a positive control. After incubation for another 4 days, the medium supernatant was removed, following which the cells were fixed according to the Adipogenesis Assay Kit and stained with Oil Red O solution. Dye extraction solution was added to extract Oil Red O and was incorporated in the fat droplets. The absorbance at 520 nm was measured using a microplate reader (SpectraMax M2e, Molecular Devices Co., USA). Each experiment was performed thrice.

The fat accumulation rate was calculated from the absorbance of each sample relative to the absorbance of the untreated control using the following formula:

$$\text{Fat accumulation rate (\%)} = (S - BL_{Avr}) / (C - BL_{Avr})_{Avr} \times 100 \quad (2)$$

where S is the absorbance of the sample solution, BL_{Avr} is the average absorbance of the progenitor cells (undifferentiated), C is the absorbance of the untreated control, and $(C - BL_{Avr})_{Avr}$ is the average value obtained by subtracting BL_{Avr} from C.

Lipid Metabolism Study on Mice

Experimental Animals and Breeding Methods

Six-week-old male ICR mice were used. The mice were fed an MF diet (Oriental Yeast Co., Ltd., Japan) for 7 days before the study commenced. They were assigned to four groups (n = 7) such that the average body weight of each group was similar before the start of the study. Each group was then fed a specific high fat (HF) diet of AIN76 powdered feed (CLEA Japan, Inc., Japan), in which a portion of the sucrose was replaced with 20% oil and fat (oil source: lard + corn oil). The AIN76 powdered feed without added fats and oils was used as the Control diet for the control group. The HFWP 0.5 and HFWP 1 groups were fed high-fat diets in which a portion of the sucrose was replaced with 0.5 and 1% (w/w) WCP, respectively. The control group was fed ad libitum, while the HF, HFWP0.5, and HFWP1 groups were fed restricted (pair

feeding). After 28 days of rearing, the animals were euthanized by cervical dislocation and blood release from the carotid artery, following which adipose tissue, liver, and blood samples were collected.

Analysis of Plasma and Liver Components

Lipids in the liver were extracted using a previously reported method [18], and the total lipid content was determined. Triglyceride E-test-Waco (FUJIFILM Wako Pure Chemicals Co., Japan) was used to extract triacylglycerols in the liver and blood plasma, cholesterol E-test-Waco (FUJIFILM Wako Pure Chemicals Co., Japan) for total cholesterol, NEFA C-Test-Waco (FUJIFILM Wako Pure Chemicals Co., Japan) for free fatty acids in plasma. The activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured using the respective kits (FUJIFILM Wako Pure Chemicals Co., Japan). Mouse plasma leptin was measured using the Mouse Leptin Assay Kit (Morinaga Institute of Biochemistry Co., Ltd., Japan), and plasma adiponectin was measured using Levis Polymer Adiponectin-Mouse/Rat (Shiba-Yagi Co., Ltd., Japan).

Effect of Water Chestnut Polyphenols on Human Body Fat

Study Participants

The participation criteria for the study participants were adults between the ages of 20 and 70 who lived or worked in Kanzaki-shi, Japan, and who wished to improve their obesity or visceral fat. Applicants were fully informed of the purpose and methods of the study, and those who gave their consent to participate in the study were administered the selection study. Of the applicants, those who met the selection criteria and did not meet the exclusion criteria were considered as participants.

Selection criteria for participants were, in principle, a BMI (kg/m^2) of at least 23 and less than 30, and a visceral fat area of at least 80 cm^2 or an abdominal circumference of at least 81 cm, referring to a previous report [19].

Exclusion criteria for study participants were those who 1) could not meet the aforementioned participation criteria (residence or place of work, age, BMI, visceral fat area or abdominal circumference); 2) had chronic diseases such as hypertension, diabetes, dyslipidemia, and renal or liver disease; 3) used drugs that may affect sugar or lipid metabolism; 4) could not stop taking dietary supplements or health foods that could affect lipid metabolism before and during the study period; 5) could not stop consuming large amounts of green tea, oolong tea, or black tea before and during the study period; 6) were pregnant, intending to become pregnant during the study period, or breast-feeding; 7) were participating in clinical trials of oral intake of other foods and medicines, or the application of cosmetics and medicines; 8) were judged by the physician to be unsuitable as subjects; 9) requested discontinuation of the study.

Test food

The test food contained 1.05 g of WCT, 0.15 g of dextrin, and 0.02 g of pullulan for a total of 1.22 g of powder and was packaged in the form of a stick. The placebo food contained 0.98 g of lactose and 0.24

g of natural coloring (brown) and was packaged in the same way as the test food [19].

Study Design

The study was a randomized, double-blind, placebo-controlled, parallel-group comparative study. The study schedule consisted of a screening trial and an 8-week main study. Forty-four people (39 males and 5 females, mean age 42.8 ± 10.2 years) who met the selection criteria according to the screening study were selected as the study participants. The study participants were separated into groups using stratified randomization based on the variables of sex, age, BMI (kg/m^2), body fat percentage (%), visceral fat area (cm^2), and abdominal circumference (cm): 22 participants were assigned to the WCT group and 22 to the placebo tea group.

Participants were asked to consume two packets of the test or placebo food mixed with 200–500 mL of hot water per day for the duration of the study: one packet immediately before breakfast (or lunch for those who did not eat breakfast) and one packet immediately before dinner. During the pre-observation period (from the cleansing period to the commencement of the main study) and the main study period, participants were instructed not to change their daily routines, such as diet and exercise habits. Furthermore, they were asked to record their consumption of WCT or placebo tea. For the examinations at weeks 0, 4, and 8, the participants were prohibited from consuming anything other than water after 9:00 p.m. on the day before the study.

Test Items

Weight, BMI, body fat percentage, abdominal circumference, and visceral fat area were measured and blood samples were drawn on the first day of intake and after 4 and 8 weeks. Dietary surveys were conducted only on the first day of intake and after 8 weeks.

Visceral fat area was measured by the bioelectrical impedance method using a visceral fat measuring device (HDS-2000 DUALSCAN, Omron Healthcare Corporation). It is reported that there is a high correlation between this method and X-ray CT image analysis [20].

Biochemical blood examination items were total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides.

For the dietary survey, the brief-type self-administered diet history questionnaire (BDHQ) was used, which estimates habitual nutrient and food intake over a period of approximately one month based on 95 items: personal characteristics (15 items), frequency and quantity of food intake (53 items), eating behavior (21 items), and others (6 items) [21, 22]. In this study, the nutrient intake values obtained from the data recorded in the BDHQs included energy, protein, fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol, carbohydrates, total dietary fiber, 6 minerals, retinol, β-carotene equivalents, retinol equivalents, α-tocopherols, 10 other vitamins, and salt equivalents. Nutrient intakes were corrected for energy intake using the density method [23].

Statistical Analysis

Data values are expressed as mean ± standard deviation. For the lipase inhibitory activity evaluation, when significant differences were found by one-way analysis of variance (ANOVA) using Excel Statistics (Social Information Service Co., Ltd.), Dunnett's test with EGCG as the control was used to test for significant differences. In the experiments examining lipid metabolism in mice fed a HF diet, if a significant difference was found by a one-way ANOVA using Excel Statistics, the Tukey–Kramer method was used to test for significant differences. In human clinical trials, two-way ANOVA (with correspondence) was used to test for significant differences in the simple main effects of group and time and in their interaction {group (WCT group, placebo tea group) × time (0, 4, 8 weeks)}. Multiple comparisons were conducted using the Tukey method for those items for which the simple main effect of time was significant. Changes in nutrient intake were tested in the same manner. Statistical analysis of the human clinical trials was performed using Excel Statistics (Social Information Service, Inc., Japan). For all testing methods, the significance level was set at $p < 0.05$.

Results and Discussion

Lipase Inhibitory Activity

The IC_{50} values of the three polyphenols contained in the water chestnut husk are shown in Table 1; trapain and eugeniin had lower IC_{50} values and exhibited stronger lipase inhibitory activities than TGG. The IC50 of WCP was 816 ± 41 mg/mL, which was converted to molar concentration and summed to 529 ± 27 μM. The IC_{50} value of WCP was slightly higher than that of TGG. Furthermore, the IC50 values of TGG and WCP were significantly higher than that of EGCG, which is known to exhibit high lipase inhibitory activity [24, 25]. In contrast, the IC_{50} values of eugeniin and trapine were slightly lower than that of EGCG, indicating their higher lipase inhibitory activity [24, 25]. The lipase inhibitory activity of EGCG is said to be influenced by the presence of pyrogallol groups in its B ring and galloyl groups in its D ring. EGCG and lipase interact with a 1:1 interaction, which is thought to alter the molecular conformation of lipase and reduce its enzyme catalytic activity [26]. Since a large number of galloyl groups are also present in the polyphenols of the water chestnut, it is likely that interaction with lipase resulted in strong inhibitory activity.

Table 1
IC_{50} values of anti-lipase activity.

Sample	IC_{50}			Unit
Purified polyphenols				
Tetra-GG	414	±	71**	μM
Eugeniin	163	±	57	μM
Trapain	141	±	7	μM
WP[a]	816	±	41	mg/ml
	529	±	27[b]**	mM
EGCG	188	±	8	μM

IC_{50} value means half-maximal (50%) inhibitory concentration for the activity of anti-lipase activity. The data are presented as mean ± standard deviation (SD, n = 3). “a” shows crude polyphenol extracted from water chestnut. “b” is the molar concentration calculated from the content of the purified components in the crude polyphenol and each molecular weight. Statistical evaluation was performed according to the Dunnett’s method. $^{**}P < 0.01$ vs. EGCG as a positive control. Since IC_{50} value of crude polyphenol was obtained by calculation, no significant difference was tested. EGCG indicates (-)-epigallocatechin gallate.

Inhibition of fat accumulation in 3T3-L1 cells

Fat accumulation was inhibited in 3T3-L1 cells by WCPs, as shown in Fig. 1. The 12.5 μg/mL WCP solution exhibited the same inhibitory effect as the 25 μg/mL EGCG solution. The total concentration of the three polyphenols in WCP was 8.1 μM, whereas the concentration of EGCG was 54.5 μM, indicating that a similar inhibition of fat accumulation was observed at approximately 1/7th of the molar concentration of EGCG. Catechins in green tea have previously been reported to inhibit fat accumulation by inhibiting the expression of the transcription factors peroxisome proliferator-activated receptor (PPARγ) and CCAAT/enhancer-binding protein (C/EBPα) in 3T3-L1 preadipocytes at an early stage of their differentiation into adipocytes [27, 28]. Furthermore, EGCG may suppress adipocyte differentiation through the insulin signaling pathway by decreasing the activities of forkhead transcription factor class O1 (FoxO1) and sterol regulatory element binding protein (SREBP1c) [29]. Inhibition of these transcription factors is also thought to be responsible for the high inhibition of fat accumulation by WCPs.

Improvement of Lipid Metabolism in Mice

Food intake, body weight gain, tissue weight, and biochemical blood profiles of mice treated with WCP are shown in Table 2. There were no significant differences between groups in body weight gain, total food intake, or liver weight during the experimental period. However, the weight of epididymal and

perirenal fat was significantly higher in the HFWP0.5 group than in the Control group but did not differ significantly from those of the HFWP1 group.

There were no significant differences between groups for total lipid weight or levels of triglycerides and cholesterol in the liver. There were also no significant differences between groups for levels of GOT and GPT, which are indicators of liver function in plasma. These findings suggest that WCPs had no effect on the liver.

The HFWP1 group was found to have significantly lower levels of plasma triglycerides than the Control and HF groups. The effects of EGCG [30], polymerized polyphenol from oolong tea [31], and resveratrol [32] on plasma triglycerides have been reported, and their mechanisms of action are considered to be lipase inhibition or the down-regulation of the expression of adipocyte differentiation-related genes. Similarly, in the present study, pancreatic lipase inhibition and fat accumulation in 3T3-L1 cells by WCP were observed in vitro.

The total cholesterol levels in plasma were significantly lower in the HFWP1 group than the HFWP0.5 group, but there was no significant difference from those of the Control or HF groups. As total cholesterol was analyzed in this study, it is necessary to specifically investigate LDL and HDL cholesterol levels in the future.

Free fatty acids were significantly lower in the HFWP0.5 group than in the Control group, which is notable as excess free fatty acids are known to induce insulin resistance [33]. In contrast, there were no significant differences in plasma adiponectin or leptin between the groups. It was considered that the increased adiponectin and leptin might suppress the production of free fatty acids; however, since there was no significant difference between the Control and HFWP1 group, further verification of the effect of WCP on free fatty acids is needed.

Effects of WCT on Human lipid Metabolism

A screening study was conducted on 44 participants who gave their consent to participate in the study, all of whom met the inclusion criteria. To conduct this study, stratified randomization was performed to allocate the participants to different groups: 22 participants each were assigned to the WCT and placebo tea groups. All participants completed the 8-week study, but 9 participants who consumed drinks and meals other than water before each examination at weeks 0, 4, and 8 were excluded at the time of analysis, resulting in 35 final participants (16 in the WCP group and 19 in the placebo tea group). Participant characteristics are shown in Table 3. There were no significant differences in age, sex, or physical measurements between the two groups.

The total polyphenol content in the WCT used in the human clinical study was 6.0 ± 0.1 g/100 g. The contents of eugeniin, TGG, and trapain in the WCT were 0.90 ± 0.02, 1.10 ± 0.02, and 0.93 ± 0.07 g/100 g, respectively. The daily polyphenol intake was 145 mg/day of total polyphenols and 22, 26, and 22 mg/day of eugeniin, TGG, and trapain, respectively.

Table 4 shows the changes from week 0 for the items involved in the prevention of metabolic syndrome, which is the objective of this study. For all items, there were no significant differences in interactions or between groups. Abdominal circumference showed a significant decrease in the main effect of time between 0 and 8 weeks in both groups. For the WCT group, visceral fat area decreased significantly between weeks 0 and 8 and between weeks 4 and 8; however, it also decreased significantly between weeks 4 and 8 in the placebo group. There were no significant differences in cross effects or main effects for body weight, BMI, or body fat percentage.

Although there were no significant differences in visceral fat areas between groups during the study period, the WCT group showed a significant decrease with each study period. This result supports the lipase inhibitory activity and fat accumulation inhibitory effect of water chestnut polyphenols in vitro and indicates that the consumption of WCT may contribute to the prevention of abdominal visceral obesity. However, since the body weights, BMIs, and body fat percentages of the participants did not change significantly, it is possible that there was a certain amount of error in the visceral fat area measurements. Although the correlation coefficient between the visceral fat measurements obtained from the device used in this study and those obtained from X-ray CT image analysis has been shown to be relatively high, it has also been shown that error increased with increasing visceral fat area for values obtained from CT image analysis [20], which may have affected the results of this study.

The results of the biochemical blood test (total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels) are summarized in Table 4. A significant increase in HDL cholesterol occurred in the WCT group between weeks 4 and 8; the results were similar in the placebo group, with no significant differences between the groups. In experiments with mice, plasma triglycerides and free fatty acids were significantly lower in the polyphenol-fed group than in the control group, but no such trend was observed in human clinical trials. In the future, it will be necessary to investigate the appropriate intake amount and study duration to clarify the detailed in vivo mechanisms.

Data from the dietary survey revealed significant increases in the levels of total dietary fiber and vitamin C in both groups and vitamin K in the placebo group. The levels of other nutrients showed little change before and after the intervention (Table S1). This suggests that participants in both groups consumed more vegetables and fruits during the clinical trial, which may have led to a decrease in abdominal and visceral fat areas and an increase in HDL cholesterol.

Conclusion

In this study, we investigated the effects of polyphenols contained in water chestnut fruits on lipid metabolism. The results showed that water chestnut polyphenol inhibited lipase activity in in vitro systems and suppressed fat accumulation in 3T3-L1 cells. However, in animal experiments with mice, the amount of fat around organs and the body weight were not reduced by water chestnut polyphenol, though triglycerides and free fatty acids in the plasma of the polyphenol-treated group were significantly lower than those in the control group. In human clinical trials, both the placebo and WCT groups showed

a significant decrease in visceral fat areas and abdominal circumferences during the intake period, but no significant difference was observed between the groups. These results suggest that there is a possibility that WCT polyphenol has a preventive effect on the onset of metabolic syndrome. However, further studies are required to elucidate the mechanisms and determine the intake amounts required for successful prevention or treatment of metabolic syndrome.

Abbreviations

ANOVA: analysis of variance

BDHQ: brief-type self-administered diet history questionnaire

DMEM: Dulbecco's Modified Eagle's Medium

EGCG: (−)-epigallocatechin gallate

FBS: fetal bovine serum

FCS: fetal calf serum

GOT: glutamic oxaloacetic transaminase

GPT: glutamic pyruvic transaminase

HDL: high-density lipoprotein

HF: high fat

HPLC: high-performance liquid chromatography

LDL: low-density lipoprotein

TGG: 1,2,3,6-tetra-O-galloyl-β-D-glucopyranose

WCP: water chestnut polyphenol

WCT: water chestnut tea

Declarations

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Author Contributions Midori Yasuda – conceptualization, methodology, validation, investigation, resources, funding acquisition, writing (original draft and review & editing); Kenichiro Yasutake –

methodology, validation, investigation, writing original draft (the human clinical studies); Daichi Oikawa – methodology, validation, investigation, writing original draft (the experiments on animals); Iori Yoshinaga and Kanako Nakashima – investigation and statistical analysis (the human clinical studies); Ai Mitsuta – statistical analysis (the experiments on animals); Madoka Saiki, Yuki Kishikawa and Hiroya Miyabara – investigation (the human clinical studies). All authors have read and agreed to the published version of the manuscript.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval Animal experiments were approved by the "Nagasaki University Animal Experiment Committee" (Approval No.: 1408271169), and the handling of mice during experiments was conducted in accordance with the "Animal Protection Law" of Japan, "Standards for Breeding, Storage and Pain Reduction" of the Ministry of the Environment of Japan, "Basic Guidelines for Animal Experimentation" of the Ministry of Education, Culture, Sports, Science and Technology of Japan, and "Nagasaki University Animal Experiment Regulations" of Nagasaki University. The human clinical trials were conducted in accordance with the "Animal Welfare Law" of Japan. In addition, human clinical studies were conducted in compliance with the Declaration of Helsinki and after review and approval by the Ethics Committee of Nishikyushu University (approval number: 19SSYH12). Prior to the start of the study, the study plan was registered with the University Hospital Medical Information Network Clinical Trials registry (UMIN000038165, October 4, 2019).

Consent to Participate For the human clinical studies, the purpose, methods, and handling of information were explained to the participants. When the participants agreed to the content of the study, they signed a consent form them. However, the participants were allowed to withdraw their consent at any time using the consent withdrawal form.

Consent for Publication All authors agreed to publish the results in Plant Foods for Human Nutrition.

Conflict of Interest The authors have no conflicts of interest that are relevant to the contents of this article.

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Tables

Tables 2 to 4 are available in the Supplementary Files section.

Figures

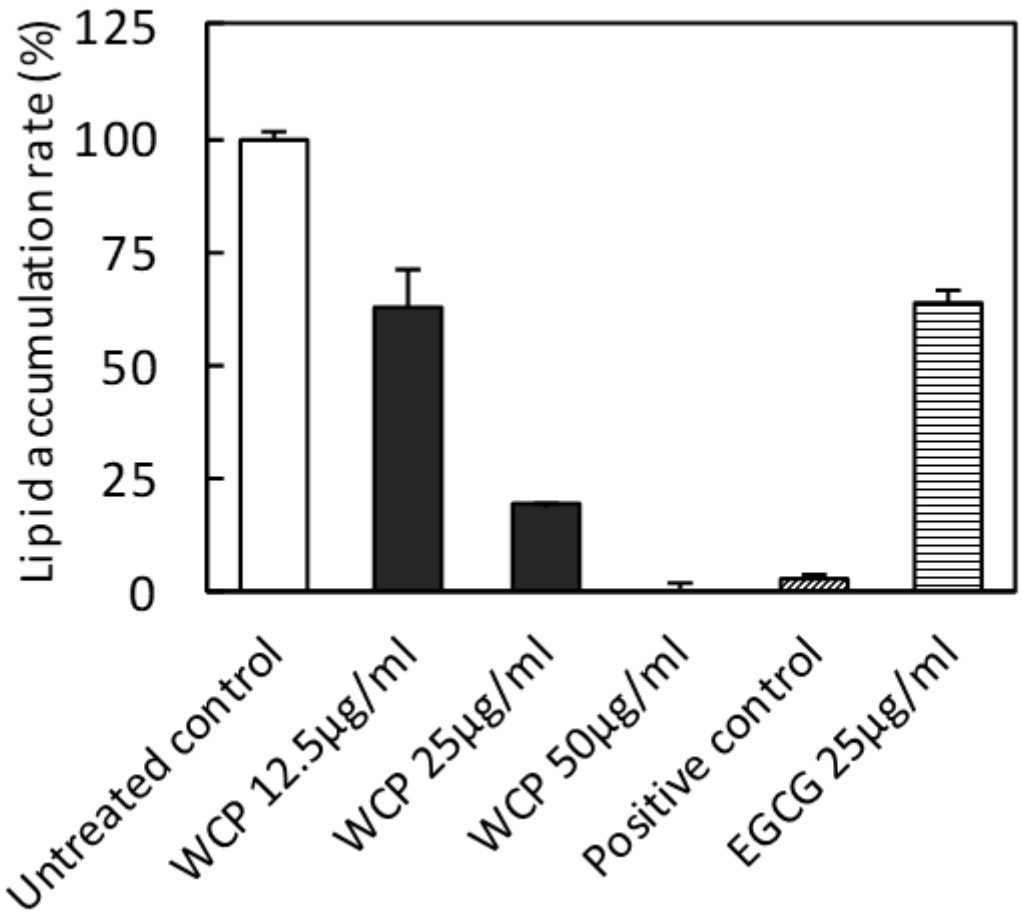


Figure 1

Inhibitory effect of water chestnut polyphenol on fat accumulation in 3T-L1 cells

WCP, positive control and EGCG represent water chestnut polyphenol, berberine chloride (1 μg/mL) and (-)-epigallocatechin gallate. Bars in the figure indicate standard deviations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable.pdf
- Table2.docx
- Table3.docx
- Table4.docx