

# Anti-obesity role of adzuki bean extract containing polyphenols: *in vivo* and *in vitro* effects

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## Abstract

**BACKGROUND:** The aim of this work was to evaluate the effects of polyphenol-rich adzuki bean extract on lipid metabolism, triglyceride accumulation and proinflammatory cytokine secretion *in vivo* and *in vitro*.

**RESULTS:** For the *in vivo* study, rats were divided into four groups: group C was fed a control diet, group A was fed the control diet with 1% adzuki bean extract, group CF was fed a high fat diet, and group AF was fed a high fat diet with 1% adzuki bean extract. For the *in vitro* study, the ability of adzuki bean extract to suppress triglyceride incorporation, glycerol phosphate dehydrogenase activity and inflammatory response was investigated in cultured human adipocytes. Data from the animal study showed that adzuki bean extract improved lipid metabolism in both the normal and high-fat diet groups. Adzuki bean extract treatment in the high-fat group resulted in significant reductions in total hepatic lipid accumulation and lipid secretion into the feces. Incubation of adipocytes with adzuki bean extract significantly decreased triglyceride accumulation, glycerol phosphate dehydrogenase activity and inflammatory responses without affecting cell viability.

**CONCLUSION:** The results of this study demonstrate that adzuki bean extract has high potential to serve as a natural anti-obesity agent.

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**Keywords:** Adzuki bean; Polyphenols; Anti-obesity

## INTRODUCTION

Obesity is defined as having a body mass index greater than 30 kg m<sup>-2</sup> and has been recognised as a global epidemic with a myriad of detrimental health effects.<sup>1</sup> It is associated with a chronic inflammatory state that has a crucial role in metabolic disorders such as hyperlipidaemia, type 2 diabetes, cardiovascular diseases, fatty liver diseases, as well as several types of cancer.<sup>2,3</sup> Hypertriglyceridaemia, the most prevalent type of dyslipidaemia, is a common risk factor for progression of obesity and other metabolic diseases. It causes excessive fat accumulation in adipose tissue, leading to hypertrophy of adipocytes and elevated levels of adipocytokines.<sup>4</sup> Some of these adipocytokines are pro-inflammatory cytokines, such as interleukin-6 (IL-6) and plasminogen activator inhibitor-1 (PAI-1), which are considered to be key modulators linking obesity and inflammation. Due to the major role of inflammation in obesity, one approach to treatment of obesity has been to target modulators such as these using agents that can elicit an anti-inflammatory response.

A potentially rich source of natural anti-obesity agents is the adzuki bean (*Vigna angularis*), which has long been cultivated throughout East Asia. In Japan, the beans are often boiled to produce sweetened adzuki bean paste for use in confectioneries. This results in the release of several bioactive ingredients from the beans, such as polyphenols and fibres, which have traditionally

been discarded. To exploit these underutilised materials, 'adzuki bean extract' has been developed (Cosmo Foods Co., Ltd, Tokyo, Japan). Recent interest has been focused on the biological activities of phenolic compounds<sup>5,6</sup> including a cardiovascular protective effect, anti-hypertensive effect and anti-obesity effect.<sup>7-10</sup> A few studies have investigated the effects of adzuki bean-derived polyphenols on hyperglycaemia,<sup>11</sup> hypercholesterolaemia<sup>12</sup> and hypertension;<sup>13</sup> however, findings regarding the effects of dietary intake of the purified adzuki bean extract in terms of overall lipid metabolism and detailed mechanisms are limited. Taking account of previous research revealing the antioxidant effects of polyphenols, we hypothesised that dietary intake of adzuki bean

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extract containing polyphenols will result in a variety of beneficial effects on health, especially in the areas of lipid metabolism and obesity, which are major topics of public health concern worldwide.

The objective of this study was to determine the beneficial effects of adzuki bean extract on obesity-related parameters, including lipid metabolism, triglyceride (TG) accumulation and inflammatory responses, using both *in vivo* and *in vitro* systems. First, the effects of adzuki bean extract intake on serum lipids, hepatic lipids and faecal lipids were investigated in rats. Because these trials showed an association between adzuki bean intake and reduced TG absorption, the lipase inhibitory activity of adzuki extract was also investigated *in vitro*. Next, a detailed investigation was carried out using human adipocytes to determine the effects of adzuki bean extract on TG accumulation, glycerol phosphate dehydrogenase (GPDH) activity, as well as secretion of adipocytokines.

## MATERIALS AND METHODS

### Adzuki bean extract

Adzuki bean extract was supplied by Cosmo Foods. Briefly, extracts were obtained from the water that was used for simmering during the processing of sweetened adzuki bean paste. This water, which contained dissolved polyphenols and other materials, was cooled, filtered and treated with enzyme followed by evaporating, and it was further sterilised and spray dried.

The composition per 100 g of manufactured adzuki bean extract (AE) was as follows: 4.26 g moisture, 2.89 g protein, 2.26 g sodium, 436 mg phosphorus, 9.83 mg iron, 93.9 mg calcium, 1.71 g potassium, 328 mg magnesium, 5.19 mg zinc, 0.08 mg riboflavin, 9.50 g dietary water-soluble fibre (Cosmo Foods). The product contains an average of 16% polyphenols (including 470 mg anthocyanidins, 20.7 mg catechins, 2.33 mg caffeic acid, 2.62 mg ferulic acid, 44.5 mg quercetin, and 102 mg protocatechuic acid).

### Experiment 1

#### Animals and diets

Male Fischer 344 rats (7 weeks old) were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). The rats were housed individually in plastic cages with a controlled 12-h light/dark cycle. Room temperature and relative humidity were kept at  $23 \pm 1^\circ\text{C}$  and  $60 \pm 5\%$ , respectively. There were no significant differences in body weight among the study groups at the beginning of the experiment. The rats were allowed free access to food and water throughout each experiment. Body weight and food consumption were recorded weekly and daily, respectively. The experimental design was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine, and adhered to the standard principles described in the Guide for the Care and Use of Laboratory Animals.<sup>14</sup>

The rats were randomly divided into four groups of five rats each and fed specific diets for a period of 4 weeks (Table 1). Using these groups, two sets of *in vivo* experiments were conducted to evaluate the effects of dietary supplementation with adzuki bean extract on (1) a normal diet and (2) a high-fat diet. To test supplementation with a normal diet, a control group (C) was fed a control diet based on the AIN-93G semi-purified rodent diet guidelines<sup>15</sup> and the adzuki bean group (A) was fed the control diet supplemented

**Table 1.** Composition of the experimental diets used in this study (g 100 g<sup>-1</sup>)

Group	Diet			
	C	A	CF	AF
Casein	20	20	20	20
L-Cystine	0.3	0.3	0.3	0.3
Soybean oil	5	5	5	5
Mineral mixture*	3.5	3.5	3.5	3.5
Vitamin mixture*	1	1	1	1
Choline bitartrate	0.25	0.25	0.25	0.25
3-Butylhydroquinone	0.0014	0.0014	0.0014	0.0014
Sucrose	10	10	10	10
Cellulose powder	5	5	5	5
Adzuki bean extract	–	1	–	1
Lard	–	–	15	15
$\alpha$ -Cornstarch	to 100	to 100	to 100	to 100

\* AIN93G mineral and vitamin mixture.  
C, control diet; A, control diet with 1% adzuki bean extract; CF, control diet with 15% lard; AF, control diet with 15% lard and 1% adzuki bean extract.

with 1% (wt/wt) adzuki bean extract. To examine the effects of supplementation with a high-fat diet, a control high-fat group (CF) was fed the control diet supplemented with 5% (wt/wt) soy bean oil and 15% (wt/wt) lard and the high-fat adzuki bean group (AF) received the high-fat control diet supplemented with 1% (wt/wt) adzuki bean extract. At the completion of the 4-week feeding period, the rats were anaesthetised by intraperitoneal administration of sodium pentobarbital and blood samples were collected. The blood samples were collected in tubes with no anticoagulant and were left at room temperature for 2 h. The serum was then separated by centrifugation at  $1500 \times g$  for 15 min. Livers were surgically removed and washed with cold saline (9 g of sodium chloride per litre of de-ionised water), blotted dry on filter paper and weighed before freezing at  $-80^\circ\text{C}$  for later analysis. All faecal excretion from the previous 3 days was also collected and weighed.

#### Serum, hepatic and faecal lipid analysis

The total cholesterol (TC), HDL-cholesterol (HDL-C), and TG concentrations in the serum were determined enzymatically using commercially available reagent kits (assay kits for the TDX system; Abbott Laboratory Co., Irving, TX, USA). The non-HDL-cholesterol (non-HDL-C) concentration was calculated as follows:  $[\text{non-HDL-C}] = [\text{TC}] - [\text{HDL-C}]$ . Total hepatic and faecal lipids were determined by a modified Folch extraction procedure using a mixture of chloroform–methanol (2 : 1, v/v).<sup>16</sup>

#### Determination of lipase inhibition

To assess lipase activity, a reaction medium was prepared by dissolving 240 mg triolein (Sigma–Aldrich Co., Tokyo, Japan), 10 mg lecithin (Wako Pure Chemical Industries, Ltd. Osaka, Japan), and 15 mg bile acid (Sigma–Aldrich) into 27 mL of  $0.1 \text{ mol L}^{-1}$  Tris–HCl buffer (pH 7.0). Adzuki bean extract was added to the mixture at a range of concentrations (0 to  $200 \mu\text{g mL}^{-1}$ ). The reaction medium was emulsified by an ultrasonic homogeniser (VP-050; Taitec Co., Ltd., Saitama, Japan) for 10 min on ice. A  $200 \mu\text{g}$  aliquot of this mixture was pre-incubated at  $30^\circ\text{C}$  with

constant stirring at 400 rpm. Next, 100 µg of pancreatic lipase (50 µg mL<sup>-1</sup>) was added to start the enzymatic reaction and the mixture was held at 37 °C with constant stirring at 700 rpm for 30–70 min. Any oleic acids that were released via hydrolysis were then measured using the NEFA–HA assay test kit (Wako Pure Chemical Industries).

## Experiment 2

### Cell cultures

Human pre-adipocytes were obtained from ScienCell Research Laboratories (San Diego, CA, USA). The cells were cultured in visceral adipocyte culture medium ver.2 (Primary Cell Co., Sapporo, Japan) with 10 ng mL<sup>-1</sup> fibroblast growth factor (Wako Pure Chemical Industries) at 37 °C in a 5% CO<sub>2</sub> atmosphere for 3 days until the cells reached confluency. Next, differentiation of human preadipocytes was initiated by culturing the cells in adipocyte culture medium ver.2, which contains 1 µmol L<sup>-1</sup> dexamethazone (Sigma–Aldrich), 0.05 mmol L<sup>-1</sup> isobutyl-1-methylxanthine (Wako Pure Chemical Industries), and 10 µmol L<sup>-1</sup> troglitazone (Sigma–Aldrich). Fresh medium was prepared every 2 days. After 7 days of culturing, the medium was replaced with adipocyte culture medium ver.2 containing adzuki extract with different concentrations (250, 500 and 1000 µg mL<sup>-1</sup> in sterilised water). Adzuki bean extracts containing both polymerised polyphenols and unpolymerised polyphenols were tested. The control groups (CN-1 and CN-2) received fresh medium with no adzuki bean extract. The cells were incubated at 37 °C in a 5% CO<sub>2</sub> atmosphere for another 48 h, and then medium was collected for enzyme-linked immunosorbent assay (ELISA) analysis. The cells were washed twice with phosphate-buffered saline and analysed as described below.

### Quantification of triglyceride accumulation and measurement of glycerol phosphate dehydrogenase activity

Following the washes with phosphate-buffered saline, the human adipocyte cells described above were lysed with an ultrasonic homogeniser (VP-050; Taitec) at 80% output for 90 s on ice. The TG content of cultured adipocytes was determined by the TG E-Test Wako (Wako Pure Chemical Industries) according to the manufacturer's protocol. The cell lysate prepared above was dissolved in enzyme extract solution, and GPDH activity was measured by GPDH assay kit (Primary Cell Co., Sapporo, Japan) according to the manufacturer's protocol. For both TG and GPDH activity normalisation, the DNA content of each well was determined using a NanoDrop ND-2000 (Thermo Fisher Scientific K.K., Yokohama, Japan).

### Measurement of adipocytokine production

The concentrations of IL-6, PAI-1 and MCP-1 in culture medium were determined by ELISA using commercial kits (Quantiline; Immunoassay R & D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's protocols.

### Statistical analysis

Data are presented as mean ± standard error for each sample group. For experiment 1, differences between groups C versus A, and groups CF versus AF were tested for significance by Student's *t*-test ( $P < 0.05$ ). For experiment 2, significant differences among adzuki extract treated cells were determined by analysis of variance (ANOVA) with the Tukey–Kramer test ( $P < 0.05$ ; SAS Institute, Cary, NC, USA). Differences between each treatment group and the control groups (CN-1, CN-2) were tested for significance by Student's *t*-test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Experiment 1

#### Body weight and food consumption

Polyphenolic compounds have been reported to exert an inhibitory effect on body weight and food intake by decreasing protein digestibility. However, there were no significant differences in body weight gain between groups C and A, or between groups CF and AF. Likewise, total food intake between groups C and A, and between groups CF and AF was not significantly different following the 4-week feeding period.

#### Serum lipid profiles

Table 2 shows the serum total cholesterol, HDL-cholesterol, non-HDL-cholesterol and TG concentrations for all groups. The total cholesterol concentration was significantly lower ( $P < 0.05$ ) in group A (1.64 ± 0.08 mmol L<sup>-1</sup>) compared to group C (1.99 ± 0.11 mmol L<sup>-1</sup>). Group AF also showed a tendency towards lower total cholesterol (1.46 ± 0.05 mmol L<sup>-1</sup>) compared to group CF (1.69 ± 0.05 mmol L<sup>-1</sup>;  $P = 0.08$ ). The non-HDL-cholesterol concentration at week 4 was significantly lower in the groups fed adzuki bean extract (group A: 0.97 ± 0.06 mmol L<sup>-1</sup>; group AF: 0.86 ± 0.03 mmol L<sup>-1</sup>) compared with that in the control groups (group C: 1.29 ± 0.08 mmol L<sup>-1</sup>; group CF: 1.06 ± 0.07 mmol L<sup>-1</sup>). TG concentration in group A (0.43 ± 0.09 mmol L<sup>-1</sup>) was also significantly lower than that in the control group (1.08 ± 0.13 mmol L<sup>-1</sup>) at week 4. Group AF also showed a tendency towards lower TG levels (0.54 ± 0.08 mmol L<sup>-1</sup>) compared with TG levels of group CF (0.77 ± 0.08 mmol L<sup>-1</sup>;  $P = 0.1$ ).

**Table 2.** Serum total cholesterol, HDL cholesterol, non-HDL-cholesterol and triglyceride concentrations in rats fed experimental diets for 4 weeks

Cholesterol/triglyceride (mmol L <sup>-1</sup> )	Normal diet		High fat diet	
	C	A	CF	AF
Total cholesterol	1.99 ± 0.11	1.64 ± 0.08*	1.69 ± 0.09	1.46 ± 0.05 ( $P = 0.08$ )
HDL cholesterol	0.70 ± 0.03	0.67 ± 0.02	0.63 ± 0.03	0.61 ± 0.02
Non-HDL cholesterol	1.29 ± 0.08	0.97 ± 0.06*	1.06 ± 0.07	0.86 ± 0.03*
Triglyceride	1.08 ± 0.13	0.43 ± 0.09*	0.77 ± 0.08	0.54 ± 0.08 ( $P = 0.1$ )

C, control diet; A, control diet with 1% adzuki bean extract; CF, high-fat diet; AF, high-fat diet with 1% adzuki bean extract. Data are expressed as mean ± SE ( $n = 5$ ).

\* $P < 0.05$  between groups C and A, or between groups CF and AF, according to Student's *t*-test.

The results reported here are in agreement with a previous study in which the serum total cholesterol levels were reduced in Sprague–Dawley rats that were fed polyphenols derived from green tea.<sup>17</sup> Furthermore, anthocyanin, which is present in adzuki bean extract, has been shown to reduce plasma total cholesterol and TG levels in rats.<sup>18</sup> Nishi and others<sup>19</sup> also reported that polyphenols derived from adzuki beans improved lipid metabolism in mice fed high-fat diets. The authors suggested that polyphenols have an inhibitory effect on the activity of acetyl-CoA carboxylase, an enzyme that leads to the production of malonyl-CoA for the biosynthesis of fatty acids. This mechanism may explain the reduced plasma TG levels in rats fed adzuki bean extract in the current study. The polyphenols in adzuki bean extract are also known to contain catechins, which can reduce cholesterol levels by inhibiting cholesterol biosynthesis. It has been suggested that catechins inhibit cholesterol absorption by interfering with the biliary micelle system in the lumen of the intestine. As a result, the amount of bile acids that return to the liver decreases, leading to an increase in the hepatic synthesis of bile acids from cholesterol and a higher expression of LDL receptors in the liver, ultimately resulting in reduced serum cholesterol concentrations.<sup>20</sup> With this in consideration, the polyphenols in the adzuki bean extract used in this study may have played an essential role in the improved lipid metabolism observed in this study.

#### Hepatic and faecal weights and lipid concentrations

Table 3 shows the weights and total lipid concentrations of the liver and faecal matter from the rats in this study. For group A, the intake of adzuki bean extract resulted in significant increases in the faecal weight ( $1.91 \pm 0.18 \text{ g}^{-1} \text{ WW day}^{-1}$ , where WW is wet weight) with respect to the control group ( $1.40 \pm 0.10 \text{ g}^{-1} \text{ WW day}^{-1}$ ); however, there were no significant differences in the liver weight or the total hepatic lipid content. For rats on the high-fat diet, group AF exhibited significantly lower total hepatic lipid content and liver weight ( $75.9 \pm 10.8 \text{ mg liver}^{-1}$  and  $2.12 \pm 0.03 \text{ g}^{-1} \text{ WW } 100 \text{ g}^{-1} \text{ bw}$ , respectively, where bw is body weight) compared to group CF ( $142 \pm 27.4 \text{ mg liver}^{-1}$  and  $2.30 \pm 0.04 \text{ g}^{-1} \text{ WW } 100 \text{ g}^{-1} \text{ bw}$ , respectively). Correspondingly, group AF had significantly higher total faecal weight and faecal lipid excretion ( $1.89 \pm 0.15 \text{ g}^{-1} \text{ WW day}^{-1}$  and  $59.8 \pm 5.35 \text{ mg g}^{-1} \text{ WW}$ , respectively) compared with group CF ( $1.23 \pm 0.06 \text{ g}^{-1} \text{ WW day}^{-1}$  and  $34.9 \pm 5.47 \text{ mg g}^{-1} \text{ WW}$ , respectively). These results indicate that adzuki bean extract inhibits total lipid accumulation in the liver with a concomitant

excretion of lipid into the faeces, an effect that is especially apparent in rats fed a high-fat diet. Similar results have been reported for other dietary polyphenol-rich materials such as black tea.<sup>21,22</sup> The exact mechanisms involved have not yet been established, but it has been shown that polyphenols can adsorb cholesterol, bile acids and dietary lipids, thereby decreasing total hepatic lipid content and increasing faecal excretion.<sup>23</sup>

In addition, an increase in total hepatic lipids is known to promote development of atherogenic dyslipidaemia, thus providing a stimulus for increased formation and secretion of non-HDL particles. This chain of events ultimately leads to higher serum levels of TG and non-HDL cholesterol.<sup>24</sup> Therefore, the reduced levels of total hepatic lipids found in the groups fed adzuki bean extract in this study are likely associated with the reduction in serum TG and non-HDL levels mentioned above for these groups.

#### Lipase inhibitory activity

As shown in Fig. 1, the addition of adzuki bean extract resulted in a dose-dependent reduction in relative pancreatic lipase activity up to a concentration of  $50 \mu\text{g mL}^{-1}$ . The  $\text{IC}_{50}$  occurred with  $12.5 \mu\text{g mL}^{-1}$  adzuki bean extract, and lipase inhibition reached a maximum of 60% with  $50 \mu\text{g mL}^{-1}$  adzuki bean extract. There were no significant differences in relative lipase activity between  $50 \mu\text{g mL}^{-1}$  and  $200 \mu\text{g mL}^{-1}$  adzuki bean extract concentrations. Figure 1B shows changes in the concentration of oleic acid ( $\text{mEq L}^{-1}$ ) released via lipase-catalysed hydrolysis in the presence or absence of  $50 \mu\text{g mL}^{-1}$  adzuki bean extract with increasing reaction times (0–70 min). The oleic acid concentrations in the reaction mixture prepared with adzuki bean extract were significantly lower than those in the mixture without adzuki bean extract at all reaction times except 0 min. Following the 70 min reaction time, the oleic acid concentration in the reaction mixture with adzuki bean extract was significantly lower ( $0.74 \pm 0.04 \text{ mEq L}^{-1}$ ) compared to the mixture prepared without adzuki bean extract ( $2.50 \pm 0.16 \text{ mEq L}^{-1}$ ). The lower levels of oleic acid in the reaction mixture containing adzuki bean extract demonstrate an inhibitory effect that prevents the lipase-catalysed hydrolysis of the substrate (triorein).

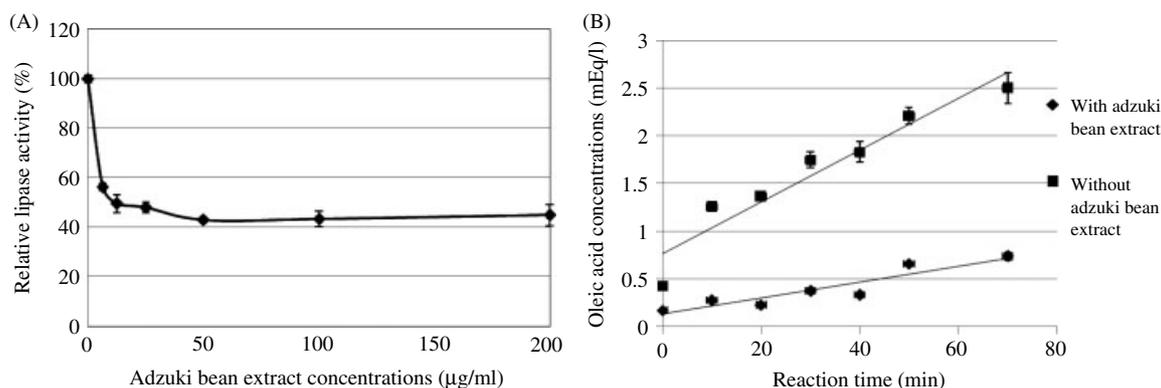
Various polyphenols have been reported to demonstrate an inhibitory effect on pancreatic lipase<sup>25,26</sup> with the greatest effects observed in polyphenols with a high degree of polymerisation. Specifically, the stereochemical structure of such polymerised polyphenols has been suggested to interfere with pancreatic lipase activity.<sup>25,27</sup> The anti-obesity effects of adzuki bean extract

**Table 3.** Weight and lipid content of liver and faecal matter in rats fed experimental diets for 4 weeks

Liver/faecal matter	Normal diet		High-fat diet	
	C	A	CF	AF
<b>Liver</b>				
Weight (wet, $\text{g g}^{-1} \text{ bw}$ )	$2.17 \pm 0.06$	$2.16 \pm 0.02$	$2.3 \pm 0.04$	$2.12 \pm 0.03^*$
Total lipid ( $\text{mg liver}^{-1}$ )	$64.4 \pm 3.86$	$66.5 \pm 6.66$	$142 \pm 27.4$	$75.9 \pm 10.8^*$
<b>Faecal matter</b>				
Weight (wet, $\text{g day}^{-1}$ )	$1.40 \pm 0.10$	$1.91 \pm 0.18^*$	$1.23 \pm 0.06$	$1.89 \pm 0.15^*$
Total lipid ( $\text{mg g}^{-1} \text{ wet wt}$ )	$22.3 \pm 1.20$	$21.3 \pm 3.22$	$34.9 \pm 5.47$	$59.8 \pm 5.35^*$

C, control diet; A, control diet with 1% adzuki bean extract; CF, high-fat diet; AF, high-fat diet with 1% adzuki bean extract. Data are expressed as mean  $\pm$  SE ( $n = 5$ ).

\* $P < 0.05$  between groups C and A, or between groups CF and AF, according to Student's *t*-test.



**Figure 1.** *In vitro* inhibition of pancreatic lipase activity by adzuki bean extract. (A) Lipase activity was determined at 37 °C and at pH 7.0 for 30 min with increasing amounts of stock solution of adzuki bean extract (0 to 200 µg mL<sup>-1</sup>). (B) The concentration of oleic acids released by lipase activity was determined at 37 °C and pH 7.0 with an increasing reaction time (0–70 min) in the presence and absence of 50 µg mL<sup>-1</sup> adzuki extract concentration. (◆), With adzuki bean extract; (■), without adzuki bean extract.

observed in our animal study with regard to reducing serum TG and total hepatic lipid concentrations with concomitant lipid excretion are likely related to the ability of the polymerised polyphenols in adzuki bean extract to inhibit lipase activity.

**Experiment 2**

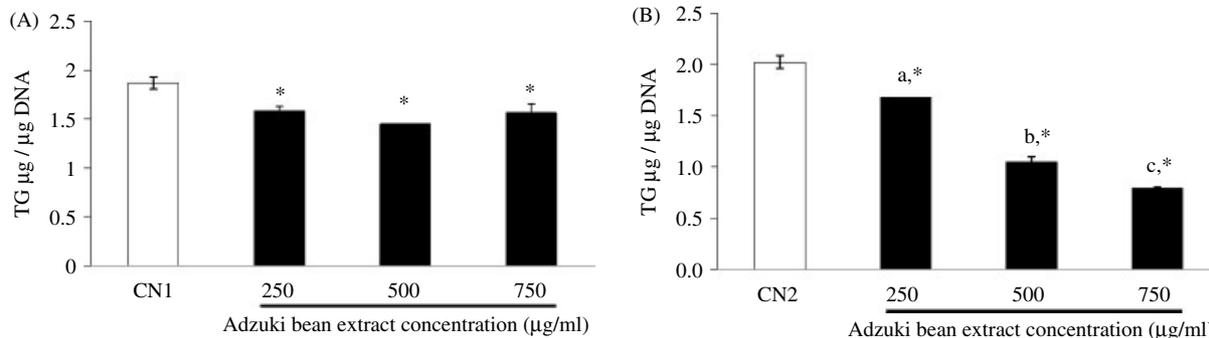
*Triglyceride accumulation in human adipocyte and glycerol phosphate dehydrogenase activity*

Figure 2 shows the effects of adzuki bean extract containing either polymerised or unpolymerised polyphenols on TG concentrations in human adipocyte cells. TG concentrations in human adipocyte cells treated with adzuki bean extract containing polymerised polyphenols were significantly lower (250 µg mL<sup>-1</sup>: 1.58 ± 0.05 µg µg<sup>-1</sup> DNA, 500 µg mL<sup>-1</sup>: 1.46 ± 0.00 µg µg<sup>-1</sup> DNA, 750 µg mL<sup>-1</sup>: 1.57 ± 0.08 µg µg<sup>-1</sup> DNA) as compared to the control (CN-1; 1.87 ± 0.06 µg µg<sup>-1</sup> DNA), although the differences were not dose-dependent (Fig. 2A). On the other hand, treatment with adzuki bean extract containing unpolymerised polyphenols resulted in a dose-dependent reduction in TG accumulation in human adipocyte cells, with adzuki extract treatments of 250, 500 and 750 µg mL<sup>-1</sup> concentrations leading to reductions in TG levels by 17, 49 and 61%, respectively, compared to the control (Fig. 2B).

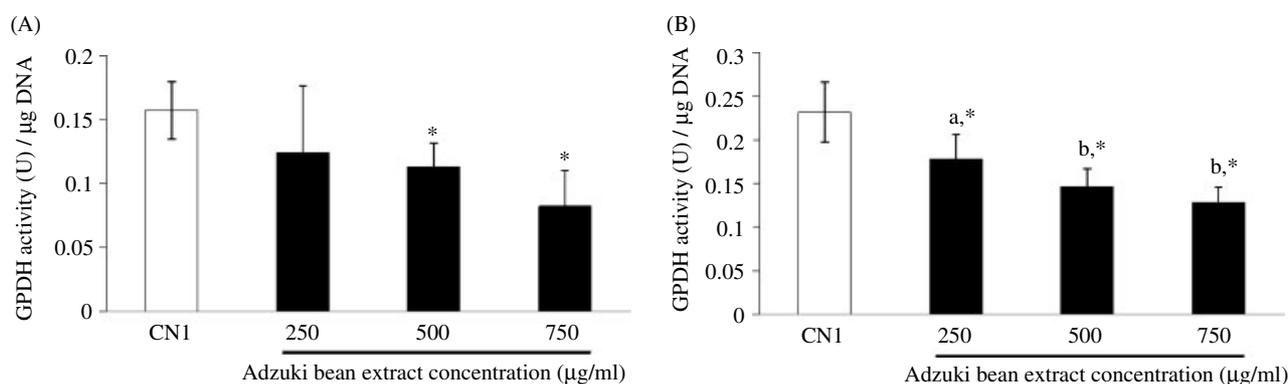
The effects of treatment with adzuki bean extract containing either polymerised or unpolymerised polyphenols on GPDH activity

in human adipocyte cells are shown in Fig. 3. Similar to the results found for TG accumulation, adzuki bean extract was found to significantly reduce GPDH activity in most cases. Although treatment with 250 µg mL<sup>-1</sup> adzuki bean extract containing polymerised polyphenols did not significantly reduce GPDH activity, cells treated with 500 and 750 µg mL<sup>-1</sup> adzuki bean extract showed significantly lower GPDH activity as compared to the control (Fig. 3A). In the case of the adzuki bean extract containing unpolymerised polyphenols, treatment with 250 µg mL<sup>-1</sup> resulted in a significant difference in GPDH activity (22% reduction compared to control and treatment with 500 µg mL<sup>-1</sup> concentration further reduced GPDH activity (35% reduction compared to the control).

Overall, both types of adzuki bean extract were able to suppress TG accumulation and GPDH activity, but the greatest effects were observed with the adzuki bean extract containing unpolymerised polyphenols. Similar to these observations, previous studies have reported that polyphenols decrease TG accumulation and stimulate lipolysis in 3T3-L1 adipocytes.<sup>28,29</sup> While 3T3-L1 cells are a well-established mouse cell line system for investigating fat metabolism, inter-species differences in adipose tissue biology are widely recognised and studies regarding effect of polyphenols using human adipocytes have been limited. However, this study demonstrated that a dietary source of polyphenols also shows important anti-obesity effects in human adipocytes, which are likely a better reflection of human lipid metabolism.



**Figure 2.** Effects of adzuki bean extract containing (A) polymerised polyphenols and (B) unpolymerised polyphenols on TG concentration in human adipocyte cells. \*Significant difference compared to control, according to a Student's *t*-test ( $P < 0.05$ ). <sup>abc</sup>A different superscript letter indicates a significant difference among cells treated with adzuki bean extract, according to ANOVA, Tukey's test ( $P < 0.05$ ).



**Figure 3.** Effects of adzuki bean extract containing (A) polymerised polyphenols and (B) unpolymerised polyphenols on GPDH activity in human adipocyte cells. \*Significant difference compared to control, according to a Student's *t*-test ( $P < 0.05$ ). <sup>ab</sup>A different superscript letter indicates a significant difference among cells treated with adzuki bean extract, according to ANOVA, Tukey's test ( $P < 0.05$ ).

### Release of cytokine and protein

Further experiments to elucidate whether adzuki bean extract had an effect on the regulation of proinflammatory cytokines (that is, PAI-1, IL-6 and MCP-1) in human adipocyte cells were very promising, with significant reductions compared to the controls for all treatment levels (Fig. 4). The greatest effects were observed with PAI-1 secretion in human adipocyte cells, where treatment with adzuki bean extract containing either type of polyphenol reduced PAI-1 concentration to  $0.03 \pm 0.02$  to  $0.05 \pm 0.05$  ng mL<sup>-1</sup> compared with  $1.80 \pm 0.41$  ng mL<sup>-1</sup> in the control. PAI-1 is generally secreted by endothelial cells, cascular smooth muscle cells, hepatocyte platelets, and adipocytes, with the majority of the circulating PAI-1 being contributed to by adipose tissue.<sup>30</sup> The heightened levels of circulating PAI-1 in obese individuals together with high plasma fibrinogen contribute to a prothrombotic state.<sup>31</sup> Furthermore, Mukai and Sato<sup>13</sup> reported that polyphenol-containing adzuki bean seed coat could attenuate vascular oxidative stress and inflammation during the progression of hypertension, and they suggested that one of the mechanisms for this may be reduction of PAI-1 secretion. This PAI-1 reduction is in agreement with the results reported here, suggesting that adzuki bean extract may attenuate progression of hypertension in addition to its anti-obesity effects.

In the case of IL-6 and MCP-1, treatment with adzuki bean extract resulted in significant reductions in the concentrations of both compounds, with the greatest effects observed for adzuki bean extract containing polymerised polyphenols. For example, IL-6 concentrations were reduced by 24–51% following treatment with adzuki bean extract containing polymerised polyphenols compared with 14–42% following treatment with the extract containing unpolymerised polyphenols. Other cytokines, such as tumour necrosis factor- $\alpha$  and resistin, were not detectable for all samples including the control because of detection limits (data not shown). IL-6 is a cytokine that provokes a broad range of cellular and physiological responses. It is known to have direct effects on cellular metabolism, leading to hypertriglyceridaemia *in vivo* by stimulating lipolysis and hepatic TG secretion.<sup>32</sup> The reduced levels of IL-6 resulting from treatment with adzuki bean extract may explain the reduction in TG accumulation and GPDH activity also observed in this study. In addition, mRNA expression of IL-6 in adipocytes treated with adzuki bean extract containing unpolymerised polyphenols was also reduced (data not shown), suggesting that the adipocyte inflammatory response system

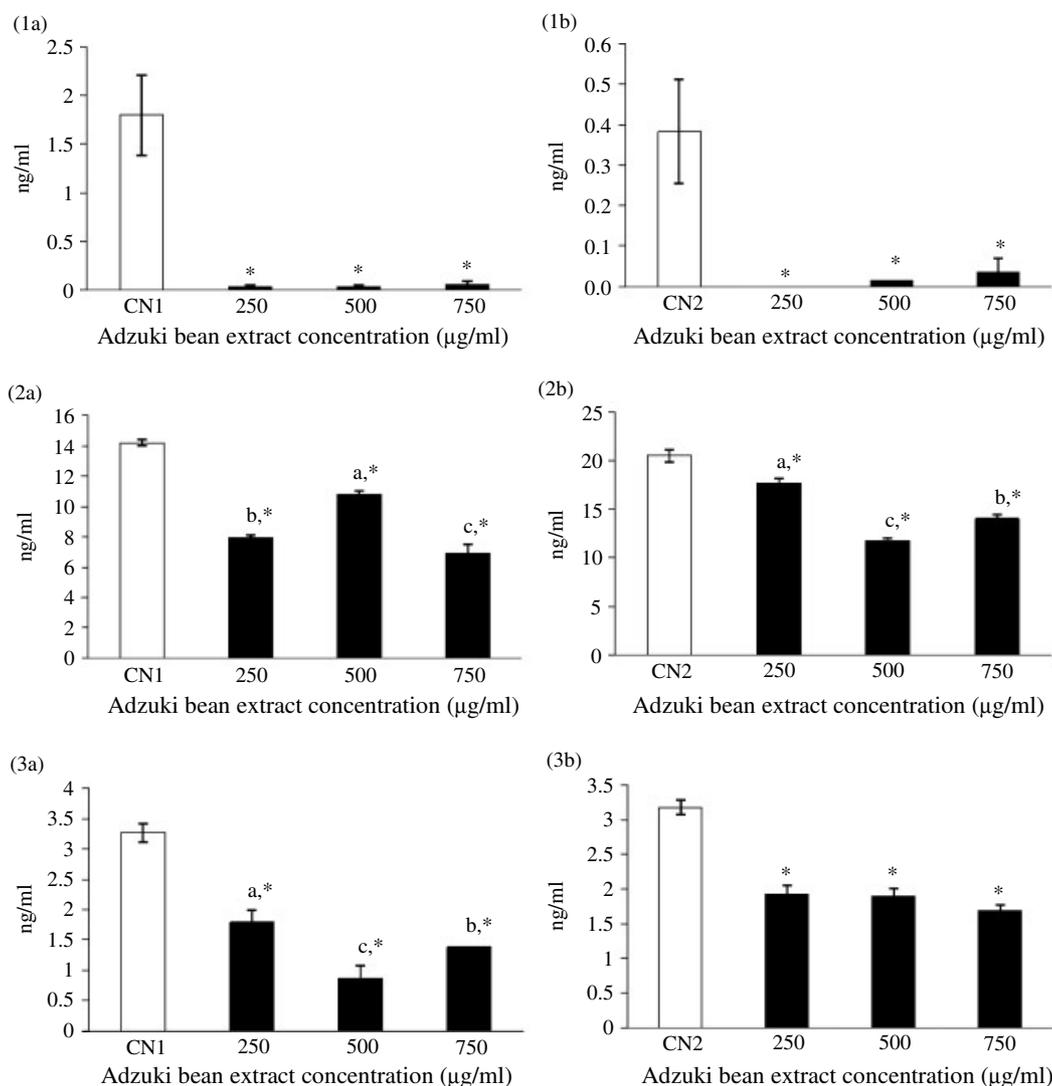
was attenuated. MCP-1, a member of the chemokine family, is another proinflammatory cytokine expressed by a variety of activated cells (e.g. endothelial cells, monocytes and smooth muscle cells). A previous study reported that prolonged exposure to MCP-1 promoted insulin resistance in differentiated adipocytes and that levels of the MCP-1 protein and mRNA expression are increased in obese mice.<sup>33</sup> As obesity is associated with an increased risk of developing insulin resistance and type 2 diabetes, MCP-1 in adipocytes likely plays an important role in altering adipocyte function and metabolism.<sup>34</sup> In our study, MCP-1 concentrations were significantly reduced by treatments with both types of adzuki bean extract, indicating that this extract may help to prevent insulin resistance and reduce the risk of type 2 diabetes.

### CONCLUSIONS

The increase in obesity-related diseases over the past several decades has heightened the need to understand the cellular and molecular mechanisms underlying lipid metabolism. By understanding these mechanisms, approaches to prevention and treatment of obesity and related diseases can be improved. In this study, adzuki bean extract demonstrated a number of beneficial anti-obesity effects by disrupting lipid metabolism, lipid accumulation, and adipocytokine production in animal and human adipocyte cultures. Specifically, the *in vivo* portion of this study demonstrated that adzuki bean extract can effectively inhibit lipid accumulation and improve lipid metabolism. Treatment with adzuki bean extract in human adipocytes also led to a reduction in TG accumulation with a simultaneous reduction in proinflammatory adipocytokines. Overall, the anti-obesity effects of adzuki bean extract demonstrated here make it a potentially important dietary supplement for the prevention and attenuation of obesity and related disorders. However, further research, including human studies, to elucidate the mechanisms of action and the relative influence of different fractions of polyphenols contained in adzuki bean extract on biological functions is needed.

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**Figure 4.** Effects of adzuki bean extract containing (a) polymerised polyphenols and (b) unpolymerised polyphenols on the secretion of (1) PAI-1, (2) IL-6, and (3) MCP-1 in human adipocyte cells. \*Significant difference compared to control, according to a Student's *t*-test ( $P < 0.05$ ). <sup>abc</sup>A different superscript letter indicates a significant difference among cells treated with adzuki bean extract, according to ANOVA, Tukey's test ( $P < 0.05$ ).

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