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# Teasaponin reduces inflammation and central leptin resistance in diet-induced obese male mice

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## Publication Details

Yu, Y., Wu, Y., Szabo, A., Wu, Z., Wang, H., Li, D. & Huang, X. (2013). Teasaponin reduces inflammation and central leptin resistance in diet-induced obese male mice. *Endocrinology*, 154 (9), 3130-3140.

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# Teasaponin reduces inflammation and central leptin resistance in diet-induced obese male mice

## Abstract

Chronic inflammation is involved in the pathogenesis of obesity and type 2 diabetes. Recently, teasaponin, an extract from tea, has been shown to have anti-inflammatory effects. We examined the effect of teasaponin on obesity, inflammation, glucose metabolism and central leptin sensitivity, in obese mice fed a high-fat (HF) diet for 16 weeks. Intraperitoneal injections of teasaponin (10mg/kg, daily) for 21 days significantly decreased the food intake and body weight of HF diet-induced obese mice. Teasaponin treatment also reduced the protein levels of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6 and/or IL-1 $\beta$ ) and NF- $\kappa$ B signaling (p-IKK and p-I $\kappa$ B $\alpha$ ) in adipose tissue and the liver. The anti-inflammatory effects of teasaponin were associated with improved glycemic status in the treated animals, evidenced by improved glucose tolerance, homeostasis model assessment (HOMA) and fasting plasma insulin. In the hypothalamus teasaponin decreased both pro-inflammatory cytokines and inflammatory signaling in the mediobasal hypothalamus. Teasaponin treatment also enhanced the anorexigenic effect of central leptin administration, restored leptin p-STAT3 signaling in the Arc, and increased hypothalamic expression of the anorexigenic peptide proopiomelanocortin (POMC). These results identify a potential novel application for teasaponin as an anti-obesity and anti-inflammatory agent.

## Keywords

obese, induced, diet, resistance, leptin, central, mice, inflammation, male, reduces, teasaponin

## Disciplines

Medicine and Health Sciences

## Publication Details

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## Teasaponin reduces inflammation and central leptin resistance in diet-induced obese male mice

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Chronic inflammation is involved in the pathogenesis of obesity and type 2 diabetes. Recently, teasaponin, an extract from tea, has been shown to have anti-inflammatory effects. We examined the effect of teasaponin on obesity, inflammation, glucose metabolism and central leptin sensitivity, in obese mice fed a high-fat (HF) diet for 16 weeks. Intraperitoneal injections of teasaponin (10mg/kg, daily) for 21 days significantly decreased the food intake and body weight of HF diet-induced obese mice. Teasaponin treatment also reduced the protein levels of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6 and/or IL-1 $\beta$ ) and NF- $\kappa$ B signaling (p-IKK and p-I $\kappa$ B $\alpha$ ) in adipose tissue and the liver. The anti-inflammatory effects of teasaponin were associated with improved glycemic status in the treated animals, evidenced by improved glucose tolerance, homeostasis model assessment (HOMA) and fasting plasma insulin. In the hypothalamus teasaponin decreased both pro-inflammatory cytokines and inflammatory signaling in the mediobasal hypothalamus. Teasaponin treatment also enhanced the anorexigenic effect of central leptin administration, restored leptin p-STAT3 signaling in the Arc, and increased hypothalamic expression of the anorexigenic peptide proopiomelanocortin (POMC). These results identify a potential novel application for teasaponin as an anti-obesity and anti-inflammatory agent.

**O**besity has reached epidemic proportions and is an important risk factor for the development of type 2 diabetes, cardiovascular disease, and some forms of cancer. There is compelling evidence that a large component of obesity-associated pathophysiology may stem from the low-grade inflammation that occurs during obesity. This includes increased production of proinflammatory cytokines such as tumor necrosis factor (TNF) alpha (TNF- $\alpha$ ), interleukin 1 beta (IL-1 $\beta$ ) and interleukin 6 (IL-6), and activation of the nuclear factor  $\kappa$ -light-chain-enhancer of activated B cells (NF- $\kappa$ B) inflammatory signaling pathway in adipose tissue, liver and the hypothalamus of the brain (1, 2).

Overnutrition induces inflammatory responses in pe-

ripheral metabolic tissues, including the infiltration of proinflammatory cytokine secreting macrophages into the adipose tissue of obese mice and humans (1, 3, 4). Proinflammatory cytokines decrease insulin sensitivity in insulin target cells (adipocytes, hepatocytes and myocytes) and contribute to systemic insulin resistance, glucose intolerance and the development of type 2 diabetes. In obese rodents fed a high-fat (HF) diet, macrophages also infiltrate the liver and increase the mRNA expression of the proinflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$  and IL-6 (5, 6). Inflammatory cytokines released by liver macrophages can activate the NF- $\kappa$ B signaling pathway in hepatocytes, causing hepatic insulin resistance (1, 6). Recent research has identified a similar type of low-grade inflammation in

ISSN Print 0013-7227 ISSN Online 1945-7170  
Printed in U.S.A.  
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Received March 6, 2013. Accepted June 3, 2013.

Abbreviations:

the hypothalamus of rodents (2), where TNF- $\alpha$ , IL-6 and I $\kappa$ B kinase (IKK) mRNA expression are increased within a week of starting a HF diet. IKK along with inhibitor kappa B alpha (I $\kappa$ B $\alpha$ ) are components of the NF- $\kappa$ B signaling pathway, and commonly used to investigate inflammatory signaling events. Hypothalamic inflammation results in central leptin resistance, hepatic insulin resistance, a reduction in thermogenesis and cardiovascular disorders (7). Therefore, blocking the peripheral and central inflammation induced by HF diet has the potential to treat obesity and metabolic syndrome. This could be achieved using novel therapies incorporating effective natural agents, particularly agents with the dual properties of preventing inflammation and controlling body weight.

From ancient times tea has been widely used as a healthy drink worldwide. Recent evidence has emerged that tea can prevent obesity and abnormal glucose and lipid metabolism (8, 9). The earliest use of tea for medicinal purposes occurred in China, in roughly 2700 BC during the time of Emperor Shen Nung (10). During that time, it was believed to have health promoting properties, and was frequently used as a fluid supply for patients with infectious diseases. Recently, the effect of tea on inflammation has received increasing attention. Six cups of green tea daily for three to eight weeks significantly reduced NF- $\kappa$ B signaling in men with prostate cancer prior to undergoing prostatectomy, compared to the control group (11). Treatment of genetically obese (ob/ob) mice with 1% tea extract in diet for 6 wk decreased hepatic TNF- $\alpha$  protein, adipose TNF- $\alpha$  mRNA, and attenuated hepatic steatosis (12). Furthermore, supplementation of HF diet with tea extract significantly reduced HF diet induced p-I $\kappa$ B $\alpha$  and NF- $\kappa$ B activity in liver and adipose tissue (13). Phenolics and saponin are the two major active components of tea extract. Phenolics in tea have been widely investigated in previous studies, while teasaponin (TS), an important bioactive ingredient of tea extract, has received little attention. Teasaponin chemically, belongs to the oleanane-type pentacyclic triterpene saponins, which are naturally derived inhibitors of NF- $\kappa$ B signaling and have anti-inflammatory potential (14). Recently teasaponin (10 mg/kg ip injection) showed significant anti-inflammatory properties, by inhibiting paw edema induced by carrageenan in rats (15). In this study, we investigated whether chronic treatment with teasaponin could reduce inflammation in the hypothalamus and peripheral tissues, and therefore improve blood glucose control and central leptin sensitivity in HF diet-induced obese mice.

## Materials and Methods

### Animals

Animals: C57Bl/6J male mice (10 wk old, body weight:  $19.62 \pm 1.40$ g) were obtained from the Animal Resources Cen-

tre (Perth, Western Australia). The mice were housed in environmentally controlled conditions (temperature 22°C, 12 h light/dark cycle). Lab chow served as the low-fat (LF) control diet (5% fat, Vella Stock Feeds, Doonside, NSW, Australia) and was provided ad libitum except where noted. All procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia, and complied with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*.

### Teasaponin treatment of high-fat diet-induced obese mice

Mice were fed standard laboratory chow for the first week to allow them to adapt to their new environment, and then placed on a high-fat (HF) diet containing 40% fat by calories (SF11-095; Specialty Feeds, Western Australia) for 16 wk. After this feeding period the mice were obese (mean body weight  $44.18 \pm 2.60$ g). The animals were then randomized into two groups, with either teasaponin (TS, 10 mg/kg) or vehicle (saline) administered daily via intraperitoneal (ip) injection for 21 d (chronic treatment of teasaponin). Food intake and body weight were recorded daily. Also included in this study were low-fat (LF) diet control mice, which were age-matched, maintained on the LF control diet, and not treated with TS. Teasaponin (96%, C<sub>57</sub>H<sub>90</sub>O<sub>26</sub>, MW = 1200) was purchased from the Aladdin Chemistry Co. Ltd, China.

Blood glucose tolerance tests were performed on day 18 of teasaponin treatment. The cannula implantation surgery was performed following 21 d of TS treatment. After a 5 d recovery from surgery, the first icv administration of leptin (or saline) was performed for the central leptin sensitivity test (detailed below). Following a 4 d interval, the mice received the second icv leptin injection and were sacrificed one hour later to collect tissues as in the previous study (16). Plasma, white adipose tissue, liver and brain tissue were collected and stored at -80°C for further analyses as detailed below.

Similar to the previous study (17), the frozen brain sections containing the arcuate nucleus were cut at 500  $\mu$ m ranging from Bregma -1.22 mm to -2.72 mm based on a standard mouse brain atlas (18), using a cryostat with the temperature set at -18°C. The arcuate nucleus was dissected and then collected using a Stoelting Brain Punch (#57401, 0.5 mm diameter, Wood Dale, Stoelting Co, USA) in an overlapping pattern over the third ventricle. The punched tissue principally contained arcuate nucleus, but we cannot rule out the inclusion of adjacent brain areas. Therefore, the punched tissue was named as the mediobasal hypothalamus.

### Intraperitoneal glucose tolerance test

On day 18 of the teasaponin treatment, the mice fasted overnight and were given an ip injection of glucose (0.5g/kg). Blood samples were taken from the tail vein, and glucose was measured using a glucometer (Freestyle; Abbott Diabetes Care, Alameda, CA) at 0 (fasting), 30, 60 and 120 min after glucose administration.

### Central leptin sensitivity tests

The central leptin sensitivity was examined in moderate and severely obese mice. For moderate obesity, mice were fed a high-fat diet for 8 wk followed by an acute treatment of teasaponin (10 mg/kg/day, ip) for 2 d. For severe obesity, the mice were fed a high-fat diet for 16 wk followed by a chronic treatment of teasa-

ponin (10 mg/kg/day, ip) for 21 d. Animals were maintained on a high-fat diet during both acute and chronic teasaponin treatment. The central leptin sensitivity test was performed as follows. Mice were anesthetized by isoflurane inhalation and placed in a stereotaxic device. An intracerebroventricular (icv) cannula was implanted into the right lateral brain ventricle (0.25 mm posterior and 1.0 mm lateral relative to Bregma and 2.5 mm below the surface of the skull) (18). Five days after implantation, the mice fasted for 6 h. Either leptin (0.1  $\mu$ g/3  $\mu$ l) or saline (3  $\mu$ l) was then injected into the lateral ventricle through the cannula. Food intake was measured at 1, 4 and 24 h, and body weight was measured 24 h after the leptin or vehicle injection. The accuracy of cannula implantation into the lateral ventricle was confirmed by examining the needle track on the brain sections of each animal.

### Conditioned taste aversion

We used a standard two-bottle preference paradigm (19, 20). Mice were adapted to 2 h daily access to water (available in two bottles) for 10 d. On day 11, the mice were given 0.15% saccharin in water in each bottle for the 2 h period instead of water, and then injected with either teasaponin (10 mg/kg, ip), vehicle (saline 0.3 ml, ip), or LiCl as a positive control (0.15 mol/l; 2 mmol/kg ip) ( $n = 8$ ). On days 12–13, the animals had water in both bottles, and on the test day (day 14), the mice were given one bottle containing saccharin and one containing water. The saccharin preference ratio was calculated as the amount of saccharin consumed divided by the total consumption of both liquids over 2 h.

### Measurement of plasma leptin, insulin, adiponectin, peptide YY (PYY) and monocyte chemoattractant protein (MCP-1)

Plasma leptin, insulin, PYY, and MCP-1 were measured using the mouse metabolic magnetic bead panel kit and adiponectin was determined with the mouse single plex adiponectin kit (Merck Millipore, MA, USA). The homeostasis model assessment (HOMA) of insulin sensitivity was calculated using the following formula: (fasting glucose [mmol/l] multiplied by fasting insulin [U/ml] divided by 22.5) (21).

### Histological analysis and morphometry

Epididymal adipose tissue was fixed in 10% buffered formaldehyde and embedded in paraffin. Tissue sections (5  $\mu$ m) were cut and mounted onto polysine slides. The sections were stained with hematoxylin and eosin and photographed at 100 $\times$  magnification. Using the software Image J 1.46r (<http://rsbweb.nih.gov/ij/download.html>) two fields per section and six sections per fat mass were analyzed to quantify the area and number of adipocytes.

### Western blot analysis

As described in our previous study (22), for protein extraction the frozen tissues were homogenized in a NP-40 lysis buffer. The following antibodies were used: TNF- $\alpha$  (sc-8301), IL-1 $\beta$  (sc-7884) and IL-6 (sc-7920) from Santa Cruz Biotechnology, and p-IKK (Ser176/180) (#2697), p-I $\kappa$ B $\alpha$  (Ser32) (#2859), p-STAT3 (Tyr705) (#9145) and SOCS3 (#2932) from Cell Signaling Technology (Beverly, MA). The bands corresponding to the proteins

of interest were scanned and band density analyzed using the automatic imaging analysis system, Quantity One (BioRad). All quantitative analyses were normalized to  $\beta$ -actin, based on our previous studies (22). Due to the small amount of tissue in the mediobasal hypothalamus, we used a previously described modified multistrip western blot (23), which allows the detection of multiple proteins with a smaller sample size than standard western blot (see supplementary figure 1 for details).

### qPCR for neuropeptide mRNA measurement

Total mediobasal hypothalamic RNA was extracted using the Aurum total RNA mini kit (Bio-Rad Laboratories, Hercules, CA) and reverse-transcribed to first-strand complementary DNA (cDNA) with the high-capacity cDNA reverse transcription kit (AB Applied Biosystems, California, USA) according to the manufacturer's instructions. Quantitative real-time PCR (qPCR) was performed in a 20  $\mu$ l final reaction volume using SYBR green I master in a Lightcycler 480 (F. Hoffmann-La Roche Ltd, Switzerland). Primers used are listed in supplemental Table 1. Amplification was carried out with 45 cycles of 95 $^{\circ}$ C for 10 s, 60 $^{\circ}$ C for 30 s and 72 $^{\circ}$ C for 30 s. mRNA expression levels for neuropeptides were normalized to gamma actin, which served as the internal control. Experiments were performed in triplicate. The level of expression for each gene was calculated using the comparative threshold cycle value (Ct) method, using the formula  $2^{-\Delta\Delta C_t}$  (where  $\Delta\Delta C_t = \Delta C_t \text{ sample} - \Delta C_t \text{ reference}$ ) as described previously (24, 25).

### Statistical analysis

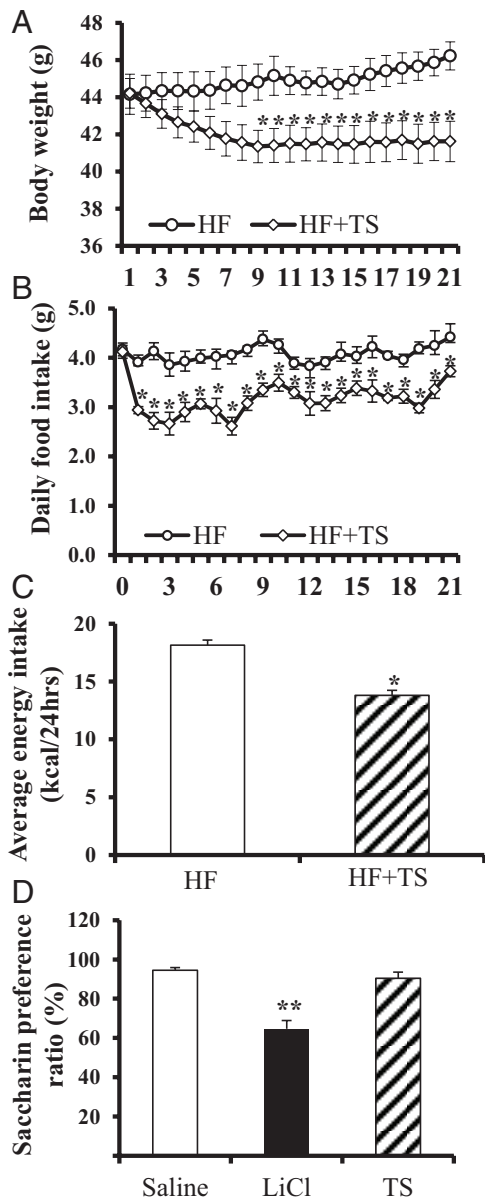
Data were analyzed using the SPSS 19 statistical package (SPSS, Chicago, IL, USA). Two-way repeated-measure ANOVA followed by two-tailed student's *t*-tests were used to analyze body weight and food intake during the teasaponin treatment. Two-tailed student's *t*-tests were used to analyze adipose tissue histology and weight, and liver weight between the teasaponin treatment and vehicle groups. One-way analysis of variance (ANOVA) followed by the post hoc Tukey–Kramer honestly significant difference (HSD) test was used to analyze the conditioned taste aversion assessment, plasma cytokines, hypothalamic neuropeptides and central leptin sensitivity test. A *p* value of less than 0.05 was regarded as statistically significant, and *p* values of less than 0.10 were considered a trend. Values are expressed as mean  $\pm$  SEM.

## Results

### Teasaponin reduced body weight and food intake in obese mice

When teasaponin was administered via ip injection to HF diet-induced obese mice it significantly reduced body weight and food intake during the 21-d observation period (Figures 1A and 1B). The body weight and food intake during the teasaponin treatment were significantly affected by the treatment, week factors and their interaction (supplementary Table 2). The final body weight and average energy intake of the teasaponin group was also significantly lower than the HF control group following





**Figure 1.** Chronic administration of teasaponin (TS, 10 mg/kg ip for 21 d) significantly decreased body weight (A), daily food intake (B) and average energy intake (C) in obese mice ( $n = 16$ ) fed a high-fat (HF) diet for 16 wk. \* $P < .05$  vs. HF control group. Panel D: Conditioned taste aversion assessment. Intraperitoneal administration of LiCl but not teasaponin (10 mg/kg ip) caused a conditioned taste aversion assessment in mice. Values are expressed as mean  $\pm$  SEM,  $n = 8$ . \*\* $P < .001$  vs. saline controls.

treatment (final body weight:  $-11.05\%$ ,  $P = .004$ ; average energy intake:  $-24\%$ ,  $P < .001$ , Figures 1A and 1C).

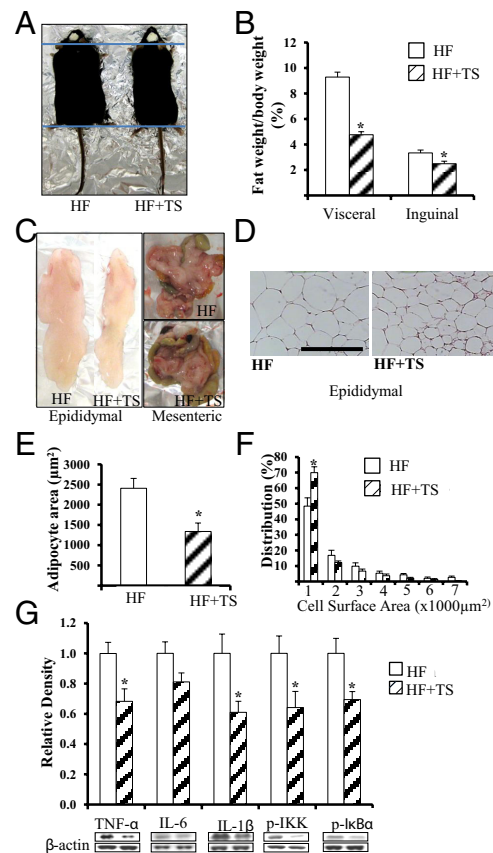
### Reduction of food intake induced by teasaponin was not associated with taste aversion

To determine whether the teasaponin induced reduction in food intake was due to taste aversion, a conditioned taste aversion test was conducted. Mice injected with LiCl consumed significantly less saccharin than the saline controls ( $P < .001$ ). In contrast, the mice that received an ip

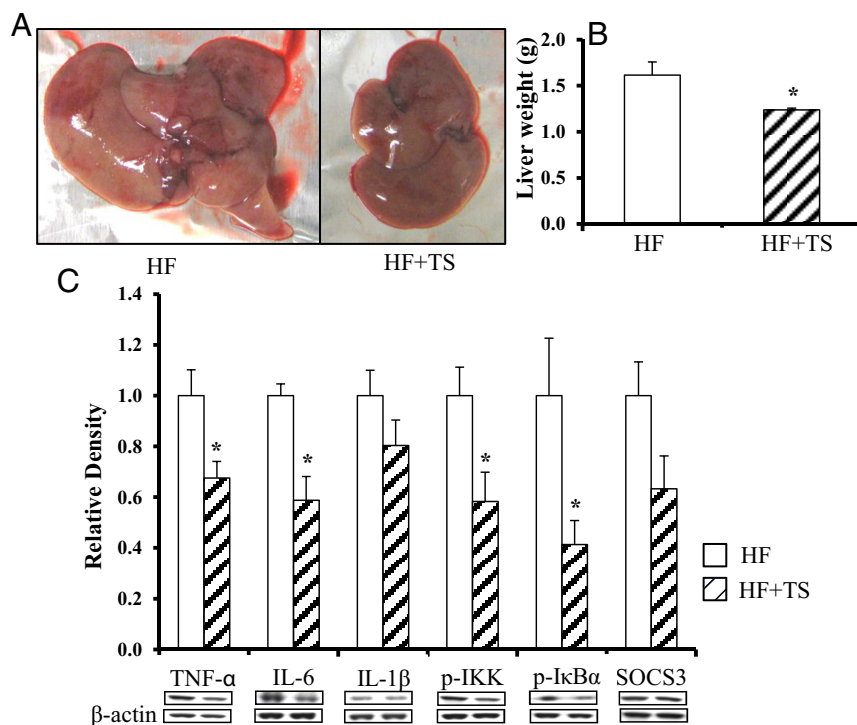
injection of teasaponin had a comparable preference for saccharin as the saline controls on the test day (Figure 1E), indicating that malaise was not a factor in the suppressive effect of teasaponin on food intake.

### Teasaponin reduced body fat and inflammation in adipose tissue of obese mice

Consistent with reduced body weight, teasaponin also significantly reduced body fat in HF diet-induced obese mice compared with the control group (Figure 2A). Compared to the controls, teasaponin treated mice had significantly lower amounts of visceral and inguinal fat as a percentage of body weight (Figure 2B). To determine whether there was a region specific fat loss, selected fat deposits were excised and weighed. The teasaponin treatment significantly decreased visceral fat, including epididymal, mesenteric and perirenal fat deposits compared with



**Figure 2.** Effect of chronic administration of teasaponin on body fat in obese mice fed a high-fat (HF) diet for 16 wk. Body size (A), and visceral fat and inguinal fat (B) were significantly decreased after TS treatment ( $n = 8$ ). Panel C: Photographs of epididymal and mesenteric fat tissue. Panel D: Hematoxylin and eosin staining of epididymal fat tissue, scale bar: 100  $\mu\text{m}$ . Panel E: Adipocyte area in epididymal fat ( $n = 8$ ). Panel F: Frequency distribution of adipocyte surface area in epididymal fat ( $n = 8$ ). Panel G: TS treatment significantly decreased protein levels of the proinflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$ , as well as the inflammatory signaling molecules p-IKK and p-I $\kappa$ B $\alpha$ , in the epididymal fat of HF diet-induced obese mice ( $n = 8$ ). \* $P < .05$  vs. HF control group.



**Figure 3.** Effect of chronic administration of teasaponin on liver appearance (A) and liver weight (B) and inflammatory markers (C) in the liver of obese mice ( $n = 8$ ) fed a high-fat (HF) diet for 16 wk. Panel C: In the liver, chronic treatment of teasaponin significantly decreased the protein levels of the proinflammatory cytokines TNF- $\alpha$  and IL-6, as well as the inflammatory signaling molecules p-IKK and p-I $\kappa$ B $\alpha$  in HF-induced obese mice. \* $P < .05$  vs. HF diet control group.

the saline controls (Table 1, Figure 2C). Teasaponin affected the size of adipocytes, with adipocytes from visceral fat pads being significantly smaller in teasaponin-treated

**Table 1.** Weight of fat deposits and muscle in high-fat (HF) diet-induced obese mice with or without teasaponin (TS) treatment.

Weight	HF	HF+TS	F value	P value
Epididymal fat (g)	1.90 $\pm$ 0.10	1.01 $\pm$ 0.06	51.650	<0.001
Mesenteric fat (g)	0.83 $\pm$ 0.06	0.45 $\pm$ 0.03	30.922	<0.001
Perirenal fat (g)	0.75 $\pm$ 0.07	0.28 $\pm$ 0.03	32.323	<0.001
Visceral fat (g)	3.49 $\pm$ 0.22	1.69 $\pm$ 0.10	50.678	<0.001
Inguinal fat (g)	1.28 $\pm$ 0.12	0.49 $\pm$ 0.03	40.275	<0.001
Soleus muscle (mg)	21.31 $\pm$ 0.77	19.50 $\pm$ 0.60	3.449	0.072

Values are means  $\pm$  SEM

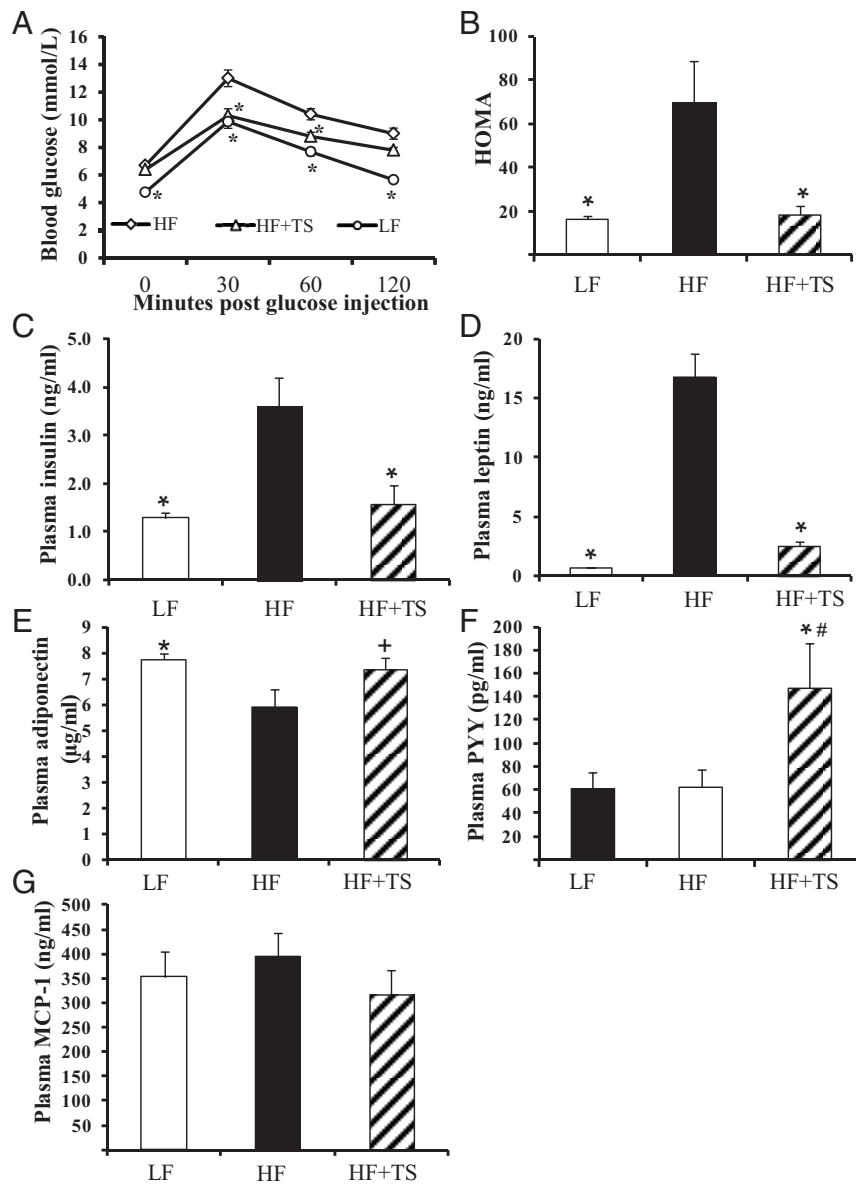
HF diet-induced mice than in the HF controls (Figures 2D and 2E). The distribution of adipocytes by cell surface area also showed a higher proportion of small sized cells ( $\leq 1000 \mu\text{m}^2$ ) in the teasaponin-treated HF group than the HF control group (Figure 2F). Finally, we found that the teasaponin treatment significantly reduced the protein levels of the proinflammatory cytokines (TNF- $\alpha$  and IL-1 $\beta$ ), as well as the inflammatory signaling molecules (p-IKK and p-I $\kappa$ B $\alpha$ ), in the adipose tissue of HF diet-induced obese mice (Figure 2G).

### Teasaponin significantly decreased hepatic inflammation

Upon gross examination we found that the teasaponin treated livers weighed less and were visibly less steatotic than the livers from the control HF diet-induced obese mice (Figures 3A and 3B). Teasaponin significantly reduced the expression of hepatic TNF- $\alpha$ , IL-6, p-IKK and p-I $\kappa$ B $\alpha$  protein in the HF diet-induced obese mice. However, teasaponin did not significantly affect the expression of hepatic IL-1 $\beta$  and SOCS3 in the same animals (Figure 3C).

### Teasaponin improved glucose tolerance and blood hormone profiles

Glucose tolerance tests were performed to assess glucose homeostasis and insulin sensitivity in HF diet-induced obese mice treated with teasaponin. Blood glucose levels were lower at the 30 and 60 min time points in the teasaponin treatment group compared to the HF controls during the glucose tolerance test (Figure 4A). The teasaponin treatment also decreased HOMA (Figure 4B), indicating that it reduced insulin resistance in the HF diet-induced obese mice. HF diet-induced hyperinsulinemia and hyperleptinemia were significantly reversed by teasaponin treatment (Figures 4C and 4D). In a statistical trend, teasaponin also reduced hypo adiponectinemia in mice induced by the HF diet ( $P = .076$ ) (Figure 4E). Circulating concentrations of the satiety peptide PYY significantly increased in the teasaponin treatment group compared with the HF and LF mice (Figure 4F). There was no significant difference in the plasma MCP-1 among the LF, HF and teasaponin-treated HF groups (Figure 4G).



**Figure 4.** Chronic administration of teasaponin improved glucose tolerance (A) and HOMA (B), decreased plasma insulin (C) and leptin (D), and increased plasma adiponectin (E) and PYY (F), in obese mice ( $n = 8$ ) fed a high-fat (HF) diet for 16 wk. Panel G: MCP-1 levels in the plasma ( $n = 8$ ). \* $P < .05$  vs. HF control group;  $^{\dagger}P < .10$  and  $> 0.05$  vs. HF group;  $^{\#}P < .05$  vs. low-fat (LF) diet control group. The homeostasis model assessment of insulin sensitivity (HOMA) is calculated by (fasting glucose [mmol/l] multiplied by fasting insulin [U/ml] divided by 22.5).

### Teasaponin increased the hypothalamic POMC mRNA expression in obese mice

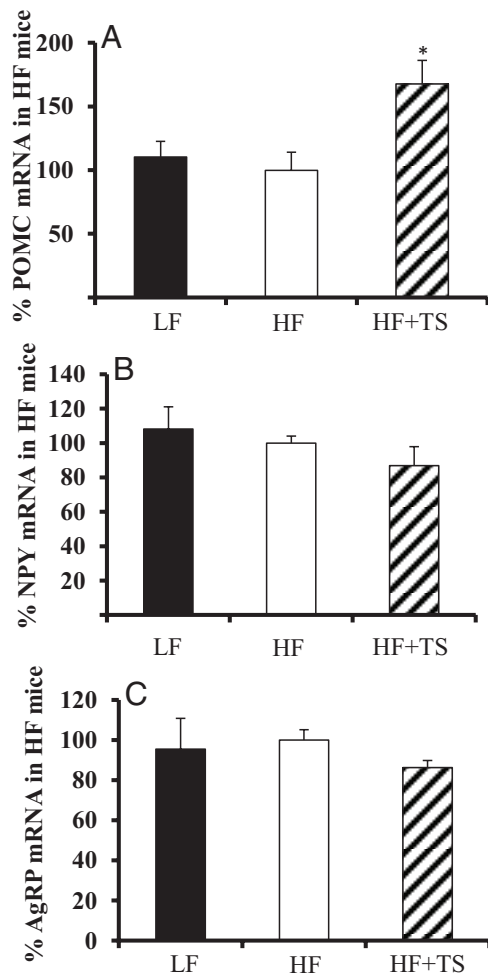
The effect of teasaponin on the expression of neuropeptides in the mediobasal hypothalamus was assessed to investigate the mechanism by which teasaponin suppresses food intake. Chronic teasaponin treatment significantly increased hypothalamic anorexigenic POMC mRNA expression compared to the HF control mice ( $P = .026$ ; Figure 5), but had no effect on orexigenic NPY and AgRP mRNA levels.

### Teasaponin attenuated hypothalamic inflammation and improved central leptin sensitivity and leptin signaling

Western blot analysis showed that in the mediobasal hypothalamus, a HF diet elevated TNF- $\alpha$ , IL-6, p-IKK and SOCS3 protein expression, which was significantly decreased by treatment with teasaponin (Figure 6). We also evaluated if leptin signaling in the CNS improved in conjunction with the observed reduction in hypothalamic inflammation. Central leptin sensitivity was examined at two stages of the obesity model, after 8 and 16 wk of HF diet, with acute and chronic teasaponin treatment respectively. After 8 wk of feeding leptin administered icv significantly decreased energy intake for 24 h in LF diet fed mice ( $-40\%$ ,  $P < .001$ ; Figure 7A), but not in HF diet fed animals ( $-16\%$ ,  $P = .421$ ; Figure 7B). In contrast energy intake was significantly decreased in the HF diet fed and teasaponin-treated mice receiving icv injections of leptin compared with the icv saline control ( $-46\%$ ,  $P = .014$ ; Figure 7B). Chronic treatment with teasaponin also improved central leptin sensitivity in mice after 16 wk of high-fat diet. Icv leptin injections did not significantly suppress energy intake in HF-induced obese mice without teasaponin treatment ( $P = .633$ ; Figure 7C), while energy intake was significantly decreased in the teasaponin-treated mice receiving icv injections of leptin compared with the icv injection of saline ( $-39\%$ ,  $P = .023$ ; Figure 7C).

To clarify the mechanisms underlying the enhanced effect of leptin in the teasaponin-treated mice, we measured p-STAT3 protein levels in the mediobasal hypothalamus after the chronic treatment with teasaponin and leptin stimulation. Leptin administered icv significantly increased p-STAT3 in LF diet fed mice ( $P < .001$ ; Figure 7D), while p-STAT3 did not significantly increase in response to the leptin injection in control HF diet-induced obese mice (Figure 7E). With the teasaponin treatment, the p-





**Figure 5.** The effect of chronic teasaponin treatment on the expression of the neuropeptide genes POMC (A), NPY (B) and AgRP (C) in the mediobasal hypothalamus of obese mice ( $n = 6-8$ ) fed a high-fat (HF) diet for 16 wk. \* $P < .05$  vs. HF group.

STAT3 level significantly increased after icv leptin injection compared with icv saline (94%,  $P < .001$ ) (Figure 7E).

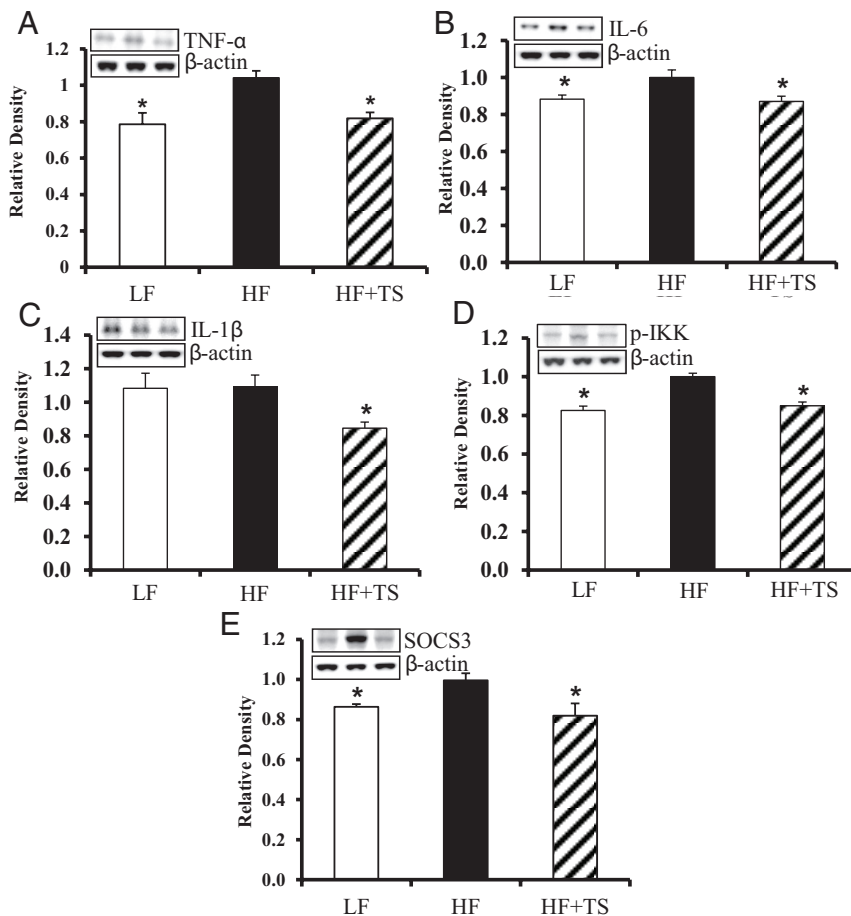
## Discussion

This study demonstrated that teasaponin treatment reduced obesity, peripheral and hypothalamic inflammation, and central leptin resistance in high-fat (HF) diet-induced obese mice. Compared with the control obese mice, the teasaponin treated obese mice had lower proinflammatory cytokines and signaling molecules in their visceral fat and liver. The anti-inflammatory effects of teasaponin were associated with an improved glycemic status in the treated animals, evidenced by improved glucose tolerance, HOMA and fasting plasma insulin. Furthermore, teasaponin decreased proinflammatory cytokines and inflammatory signaling in the mediobasal hypothalamus, and enhanced the anorexigenic effect of central leptin ad-

ministration as demonstrated by the restoration of p-STAT3 signaling in the mediobasal hypothalamus. Chronic teasaponin treatment also suppressed energy intake and increased the expression of the anorexigenic neuropeptide POMC in the hypothalamus.

A low-grade proinflammatory state is at the pathogenic core of obesity and its associated metabolic syndrome. We have demonstrated for the first time that teasaponin (ip injection) lowers proinflammatory cytokines (TNF- $\alpha$ , IL-6 and IL-1 $\beta$ ) in the adipose tissue of HF diet-induced obese mice. Adiponectin is secreted from adipose tissue and functions to reduce plasma glucose and fatty acid levels (26). Several studies have shown that obesity-associated inflammation can impair the production of adiponectin. For example, in vitro studies demonstrated that TNF- $\alpha$  and/or IL-1 $\beta$  suppressed adiponectin expression in human preadipocytes, adipose tissue and the 3T3-L1 cell line (27-29). In a human study, it was found that adiponectin plasma levels and adipose-tissue gene expression were significantly lower in obese subjects, and inversely correlated with the inflammatory markers, IL-6 and high-sensitive C-reactive protein (CRP) (30). In our study, the teasaponin treatment was found to increase the level of plasma adiponectin in HF mice, which may result from the inhibition of the proinflammatory cytokine expression in abdominal fat. The increase of plasma adiponectin after teasaponin treatment may contribute to the antidiabetogenic effect observed in this study, as treatment with adiponectin has been found to improve insulin sensitivity in obese mice (31).

The inflammatory signaling molecules p-IKK and p-I $\kappa$ B $\alpha$  were decreased in the adipose tissue and liver of obese mice treated with teasaponin. The IKK/I $\kappa$ B $\alpha$  complex mediates the activity of the NF- $\kappa$ B transcription factor to regulate the expression of various cytokines. Activation of IKK by phosphorylation induces phosphorylation and degradation of its substrate, the inhibitory protein I $\kappa$ B $\alpha$ , which is normally bound to NF $\kappa$ B. The dissociation and degradation of I $\kappa$ B $\alpha$  releases NF $\kappa$ B to translocate into the nucleus and mediate the transcription of various genes, including TNF- $\alpha$ , IL-1 $\beta$  and IL-6 (32). Mice overexpressing IKK in their hepatocytes had an increased hepatic production of IL-6, IL-1 $\beta$  and TNF- $\alpha$  (6). We have shown that teasaponin reduces the phosphorylation of IKK and I $\kappa$ B $\alpha$  in the liver and adipose tissue, indicating that teasaponin inhibits proinflammatory cytokines by suppressing NF $\kappa$ B signaling upstream of IKK/I $\kappa$ B $\alpha$ . The NF- $\kappa$ B signaling pathway affects glucose metabolism in multiple tissues (6, 33). For example, mice overexpressing IKK in hepatocytes exhibit hyperglycemia, profound hepatic insulin resistance and moderate systemic insulin resistance (6). Recently, IKK has been iden-



**Figure 6.** Effect of chronic teasaponin treatment on the protein levels of inflammatory markers in the mediobasal hypothalamus of obese mice fed a high-fat (HF) diet for 16 wk. Chronic treatment of teasaponin significantly decreased the level of the proinflammatory cytokines TNF- $\alpha$  (A), IL-6 (B) and IL-1 $\beta$  (C), as well as the inflammatory signaling molecules p-IKK (D) and SOCS3 (E).  $n = 6-8$ , \* $P < .05$  vs HF group.

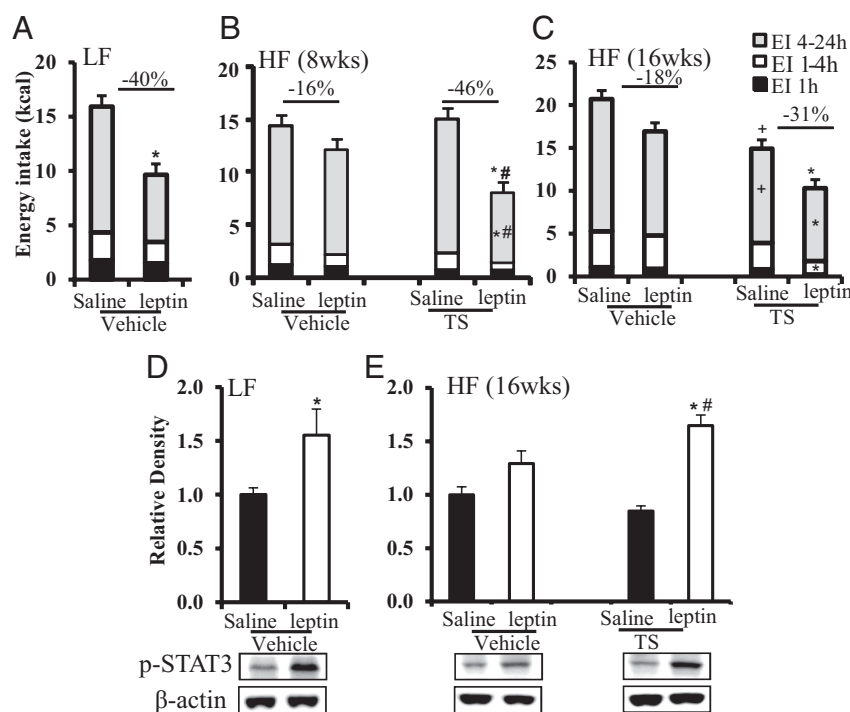
tified as a target of anti-inflammatory therapy for the treatment of obesity-associated type 2 diabetes. Hepatocyte-specific deletion of IKK improved insulin sensitivity in mice fed a HF diet (33). The pharmacological inhibition of IKK also prevented liver steatosis in obese mice (34). Thus, teasaponin represents a novel strategy to suppress IKK/I $\kappa$ B $\alpha$  complex mediated responses, and ameliorate the metabolic disorders related to diet-induced obesity.

Dietary teasaponin supplementation (0.5%) for 11 wk prevented fat accumulation and obesity in mice fed a high-fat diet by inhibiting pancreatic lipase activity, thereby delaying the intestinal absorption of dietary fat (35). In the current study, the chronic ip administration of teasaponin decreased body weight and improved many other obesity-linked parameters in mice after fed a HF diet for 16 wk. Furthermore, ip injection of teasaponin suppressed food intake in the obese mice, consistent with a previous study where the oral administration of teasaponin decreased the short-term feeding response in chicks (36). In addition, we found that suppressing food intake did not elicit malaise,

in that an effective dose (ED) of teasaponin did not cause a conditioned taste aversion. Therefore, visceral illness is unlikely to be a factor in the feeding suppressive actions of peripherally administered teasaponin. Importantly, this study found that teasaponin treatment increased the satiety hormone peptide YY (PYY) in the plasma of HF diet-induced obese mice. Peripheral infusion of PYY reduces food intake in rodents (37), and PYY transgenic mice with increased plasma PYY concentrations are protected against diet-induced obesity (38). In this study, the teasaponin induced increase in plasma PYY may contribute to the suppression of food intake and decrease in body weight in HF diet-induced obese mice. The precise mechanism by which teasaponin elevated the plasma concentrations of PYY is unclear, however it is known that PYY is released from specialized enteroendocrine cells called L cells, predominantly located in the mucosa of the distal gastrointestinal (GI) tract (39). A HF diet induces oxidative GI mucosal injury in rats (40), and teasaponin has previously been shown to have gastroprotective

effects, where the oral administration of teasaponin prevented the gastric lesions induced by ethanol in rats (41). Therefore, the prevention of gastric lesions by teasaponin may increase the PYY secretion in the GI tract of HF diet-induced obese mice.

Two types of neurons regulate energy balance in the Arc of the mediobasal hypothalamus, anorexigenic (POMC) and orexigenic (NPY/AgRP). Chronic teasaponin treatment significantly increased POMC, but not NPY/AgRP mRNA in the hypothalamic Arc of HF diet-induced obese mice, implying that teasaponin exerts its anorexigenic action at least partially by activating the POMC system. It has been reported that peripheral PYY activates anorexigenic POMC neurons in the hypothalamic Arc. Intraperitoneal injection of the truncated form of PYY (PYY<sub>3-36</sub>) increased the expression of c-fos in the POMC neurons (42, 43), expression of POMC mRNA (44), and the action potential firing rate of POMC neurons in the hypothalamic Arc (42). Therefore, the teasaponin-induced increase in POMC mRNA expression may result from ele-



**Figure 7.** Energy intake (EI) at 1, 4 and 24 h after the icv injection of leptin or saline in low-fat (LF) diet fed mice ( $n = 7-8$ ) (A), in obese mice fed a high-fat (HF) diet for 8 wk with or without acute treatment of teasaponin (TS, 10 mg/kg ip daily for 2 d) ( $n = 7-8$ ) (B), in obese mice fed a HF diet for 16 wk with or without chronic treatment of teasaponin (10 mg/kg ip daily for 21 d) ( $n = 6-8$ ) (C). STAT3 phosphorylation in the mediobasal hypothalamus 1 h after the icv injection of leptin or saline in LF diet fed mice ( $n = 7-8$ ) (D) and obese mice fed a HF diet for 16 wk with or without chronic treatment of teasaponin ( $n = 6-8$ ) (E). \* $P < .05$  vs. icv injection of saline within the treatment group (LF, HF control, or HF with TS treatment); # $P < .05$  vs. HF group (vehicle) with an icv injection of leptin; + $P < .05$  vs. HF group (vehicle) with an icv injection of saline.

vated plasma PYY levels. However, Ghamari-Langroudi et al. reported that in an electrophysiological slice study, PYY<sub>3-36</sub> inhibited the action potential firing activity of POMC neurons of the arcuate nucleus through the postsynaptic Y2 receptor (45). Therefore, the role of POMC neurons in mediating the anorexigenic response to peripheral PYY during teasaponin treatment requires further study.

Leptin promotes negative energy balance by signaling in the brain, and the hypothalamus is a key region for the control of food intake by this hormone. This negative feedback loop becomes disrupted in most obese individuals, resulting in a state known as central leptin resistance. In this study we confirmed central leptin resistance in HF diet induced obese mice. The icv injection of leptin significantly decreased food intake in LF control mice, but not in HF obese mice with hyperleptinemia. There are two mechanisms that explain central leptin resistance, hyperleptinemia (46) and hypothalamic inflammation (2, 47). Firstly, it is reported that hyperleptinemia is required for the development of leptin resistance in diet-induced obese mice (46). In the current study, teasaponin significantly reduced

body fat and dramatically ameliorated hyperleptinemia induced by HF diet-induced obesity. This may have reduced the overstimulation of the leptin receptor and downstream signaling, thus improving central leptin sensitivity. Secondly, recent studies have revealed that hypothalamic inflammation can mediate central leptin resistance in HF diet-induced obese rodents (2, 47). Constitutive activation of IKK $\beta$  in the hypothalamus of mice induced central leptin resistance and impaired leptin signaling through p-STAT3 (47). In contrast, a genetic or pharmacological blockade of inflammatory signaling in the hypothalamus improved leptin sensitivity and elevated p-STAT3 (47, 48). In mice with an IKK knockout in hypothalamic AgRP neurons, the level of p-STAT3 was significantly increased in response to icv administered leptin in animals fed a HF diet (47). In this study, teasaponin decreased the expression of the hypothalamic proinflammatory cytokines and inflammatory signaling molecules, such as TNF- $\alpha$ , IL-6, IL-1 $\beta$  and p-IKK. This

may have contributed to the improved leptin sensitivity and hypothalamic leptin signaling via p-STAT3 following the teasaponin treatment in the diet-induced obese mice.

SOCS3 has been identified as a negative regulator of central leptin signaling. The overexpression of SOCS-3 results in the inhibition of leptin signaling through JAK2/STAT3 (49). Negative feedback in response to excessive hormone stimulation is a classical mechanism of hormone resistance. It is known that leptin stimulates the expression of SOCS3, which directly inhibits leptin signaling in the hypothalamus (50). Furthermore, overexpression of IKK in the hypothalamic neurons of mice increases SOCS3 mRNA expression and protein levels in the hypothalamus (47). Therefore in the current study, hyperleptinemia and hypothalamic inflammation in diet-induced obese mice may activate a common negative regulator of leptin signaling, SOCS3, and contribute to central leptin resistance. Upregulation of SOCS3 in POMC neurons leads to impairment of STAT3 signaling, with subsequent leptin resistance, obesity, and glucose intolerance (51). In contrast, hypothalamic SOCS3-deficient rats exhibited enhanced leptin-induced STAT3 activation, decreased body weight

gain and improved metabolic parameters when exposed to a high-fat diet (52). In the present study, teasaponin significantly decreased the level of SOCS3 in the hypothalamus of HF induced obese mice, suggesting that SOCS3 is a potential target for teasaponin's therapeutic intervention during obesity.

In summary, we have demonstrated that chronic teasaponin treatment significantly reduced food intake and body weight in HF diet-induced obese mice. Teasaponin also increased the circulating concentrations of the anorexigenic hormone PYY, and stimulated the expression of the hypothalamic neuropeptide POMC. Treatment with teasaponin significantly ameliorated peripheral and central inflammation by reducing proinflammatory cytokines and inflammatory signaling molecules in the liver, adipose tissue and hypothalamus. Therefore, teasaponin has important effects in improving glucose tolerance, central leptin sensitivity and hypothalamic leptin signaling. These results identify a novel role for teasaponin as an antiobesity and anti-inflammatory agent.

## Acknowledgments

The authors wish to thank the following individuals for their contributions. A/Prof. K. Russell (Department of Applied Statistics, University of Wollongong) for his suggestions regarding the statistical analysis, Dr. Elisabeth Frank for her technical support in intracranial surgery, Ms. Linda Cohen for her editorial revision of the manuscript, and Prof. Songbai Liu, Zhejiang University of China for providing the teasaponin. This study was supported by Diabetes Australia Research Trust Research Projects to Prof XF Huang. Y.H.Y. is supported by the National Health and Medical Research Council

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This work was supported by .

Disclosure Summary: The authors of this manuscript have nothing to disclose.

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