



# Anti-obesity action of oolong tea

L-K Han\*<sup>1</sup>, T Takaku<sup>2</sup>, J Li<sup>1</sup>, Y Kimura<sup>1</sup> and H Okuda<sup>1</sup>

<sup>1</sup>2nd Department of Medical Biochemistry, School of Medicine; and <sup>2</sup>Central Research Laboratory, Ehime University, Shigenobu-cho, Onsen-gun, Ehime 791-0295, Japan

**OBJECTIVE:** Oolong tea is traditionally reported to have anti-obesity and hypolipidaemic effects. The present study was performed to clarify whether oolong tea prevented obesity induced in mice by the oral administration of a high-fat diet for 10 weeks.

**DESIGN:** High-fat diet-induced obese mice were treated with oolong tea for 10 weeks. The effects of various active fractions isolated from oolong tea on noradrenaline-induced lipolysis were examined with isolated fat cells and a cell-free system consisting of lipid droplets and hormone-sensitive lipase (HSL).

**RESULTS:** The mean food consumption was not significantly different between high-fat diet-treated mice and high-fat plus oolong tea diet-treated mice. Oolong tea prevented the obesity and fatty liver induced by a high-fat diet. A water extract of oolong tea enhanced noradrenaline-induced lipolysis, and the active substance was identified as caffeine. Caffeine enhanced noradrenaline-induced lipolysis in fat cells without a concomitant increase in HSL activity and also accelerated the hormone-induced lipolysis in a cell-free system consisting of lipid droplets and HSL, but not in the cell-free system with sonicated lipid droplets and HSL. Oolong tea extract inhibited pancreatic lipase activity.

**CONCLUSION:** It was demonstrated that the anti-obesity effects of oolong tea in high-fat diet-treated mice might be due partly to the enhancing effect of caffeine isolated from oolong tea on noradrenaline-induced lipolysis in adipose tissue, and to the inhibitory action of some other substance in oolong tea on pancreatic lipase activity. Caffeine was found to enhance lipolysis through acting on lipid droplets but not on HSL. The results suggest that oolong tea may be an effective crude drug for the treatment of obesity and fatty liver caused by a high-fat diet.

**Keywords:** oolong tea; anti-obesity; pancreatic lipase; lipolysis; fat cells

## Introduction

Three kinds of tea: oolong, green and black, have been widely used as healthy drinks from ancient times all over the world, especially to prevent obesity and improve lipid metabolism. Among the three teas, oolong tea is traditionally reported to have anti-obesity and hypolipidaemic actions. Kimura *et al*<sup>1</sup> reported that the three kinds of tea prevented the elevations of serum and liver lipids (total cholesterol and triglyceride) and liver injury with an increase in serum transaminases (glutamic pyruvic transaminase and glutamic oxaloacetic transaminase) in rats fed peroxidized oil for one week. Furthermore, we found that tea tannins such as epigallocatechin, epicatechin gallate and epigallocatechin gallate, strongly inhibited lipid peroxidation in rat liver mitochondria and microsomes.<sup>2</sup> Recently, we found that obesity was induced by feeding a high-fat diet containing 40% beef tallow for 10 weeks to female mice.<sup>3</sup> In the present study, we used a high-fat diet-induced model of obesity in mice to clarify whether oolong tea prevents obesity. In addition, we attempted to isolate

the anti-obesity effectors from oolong tea using a lipolytic assay in rat adipocytes and an inhibitor assay on pancreatic lipase.

## Material and methods

### Materials

The [<sup>3</sup>H]triolein (tri[9,10-<sup>3</sup>H]oleoylglycerol) was from Amersham Japan (Tokyo, Japan), triolein from Sigma (St Louis, MO), noradrenaline was purchased from Daiichi Pharmacy Co. (Tokyo, Japan), collagenase (type IV) was purchased from Worthington Biochemical Co. (Freehold, NJ), and bovine serum albumin (BSA) was purchased from Wako Pure Chemical Ind. (Osaka, Japan) and was extracted by the method of Chen<sup>4</sup> to remove free fatty acid. The triglyceride E test kit was purchased from Wako Pure Chemical Ind. Pancreatic lipase and tea tannins such as (–)-epicatechin, (–)-epigallocatechin, (–)-epigallocatechin gallate and (–)-epicatechin gallate were obtained from Sigma.

### Plant materials

Oolong tea produced from Fu-Jian Sheng in China was purchased from Uchida Wakan-Yak Ind. (Osaka, Japan), and voucher samples are stored at the 2nd Department of Medical Biochemistry, School of

Correspondence: \*Dr Yoshiyuki Kimura, 2nd Department of Medical Biochemistry, School of Medicine, Ehime University, Shigenobu-cho, Onsen-gun, Ehime 791-0295, Japan.  
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Medicine, Ehime University. The botanical classification of oolong tea was identified as *Thea sinensis* L. by Prof. Dr M. Kubo (Department of Natural Drug Resources, Faculty of Pharmaceutical Sciences, Kinki University, Japan) and Prof. Dr Y. Zheng (Chinese Medicine Material College, Jilin Agricultural University, China). Voucher specimens of oolong tea were matched with specimens in the botanical repository of the above two universities in Japan and China.

#### General experimental procedures

Proton and carbon nuclear magnetic resonance ( $^1\text{H}$ - and  $^{13}\text{C}$ -NMR) spectra were measured at 270 MHz and 67.8 Hz, respectively, on a JEOL GSX-270 spectrometer. High-performance liquid chromatography (HPLC) was carried out on a TOSOH AS-810 (TOSOH Co. Ltd, Japan).

#### Extraction and isolation of a lipolytic substance augmenting noradrenaline-induced lipolytic activity

The dry leaves of oolong tea (100 g) were extracted with boiling water (1 litre) for 1 h under reflux. The water solution was concentrated to give a dark brown extract (33 g) having noradrenaline-augmenting lipolytic action. A portion (5 g) of the dark brown aqueous extract was fractionated into fractions of molecular weight (MW) > 100 kDa, 30–100 kDa, 10–30 kDa, 3–10 kDa, 1–3 kDa and < 1 kDa, using Diaflo Ultrafilters (YM1 = 1000 MW, YM3 = 3000 MW, YM10 = 10 000 MW, YM30 = 30 000 MW and YM100 = 100 000 MW cut-off membrane, Amicon, Inc, Beverly, MA). The below 1 kDa MW fraction enhanced noradrenaline-induced lipolysis at a concentration of 1000  $\mu\text{g}/\text{ml}$ . The below 1 kDa MW fraction was repeatedly purified by reverse-phase HPLC as follows; column: YMC-pack ODS-AM302 (4.6 mm ID  $\times$  150 mm length), flow rate: 1.0 ml/min, eluent solvent: 10% acetonitrile containing 0.1% trifluoroacetic acid (TFA), detection: 210 nm, sample volume 100  $\mu\text{l}$  (50 mg/ml sample concentration), column temperature: 20°C. The eluate was divided into five fractions (A, B, C, D and E), and an active substance having noradrenaline-augmenting lipolytic action was isolated from fraction B by repeated chromatography using reverse-phase HPLC (10% acetonitrile containing 0.1% TFA). An active substance was concluded to be identical with an authentic sample of caffeine, based on the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data. Yield 3.39%.

#### Estimation of body, parametrial adipose and liver weights and liver triglyceride contents

Female ICR mice (three weeks old) were obtained from CLEA (Osaka, Japan), and maintained under a 12 h/12 h light/dark cycle, in a temperature- and humidity-controlled room. The animals were given laboratory pellet chow (CLEA Japan Inc; protein 24%, lipid 3.5%, carbohydrate 60.5%) and water *ad*

*libitum*. Fifty-four mice were divided into three groups ( $n = 18$  each), with the groups matched for body weight, after one week of feeding. The control mice were fed laboratory pellet chow (lab. chow). The mice of high-fat diet-fed groups received the high-fat diet (beef tallow 40%, casein 36%, corn starch 10%, sugar 9%, vitamin mixture (AIN-76) 1% and mineral mixture (AIN-76) 4% w/w per 100 g diet) and water for 10 weeks *ad libitum*. The mice of the experimental group received the high-fat diet containing 5% oolong tea powder (raw plant dry leaf) for 10 weeks. The body weight of each mouse was estimated once a week. The total amount of food intake by each mouse was recorded at least three times every week. Following over-night starvation they were killed after anaesthetization with diethyl ether for about 2 min, and the liver and parametrial white adipose tissues were quickly removed. The liver tissues were stored at  $-80^\circ\text{C}$  until analysis was performed. The liver triglyceride content was estimated as follows; a portion (0.5 g) of the liver tissue was homogenized in Krebs Ringer phosphate buffer (pH 7.4, 4.5 ml), and the homogenate (0.2 ml) was extracted with chloroform-methanol (2:1, v/v, 4 ml). The extract was concentrated under a nitrogen stream, and the residue was analyzed using a Triglyceride E-Test kit.

#### Preparation of fat cells

Young male Wistar rats (five weeks old) were obtained from CLEA (Osaka, Japan). Rats weighing 150–160 g (six weeks old) were given a standard laboratory diet (Oriental Yeast Co. Ltd, Tokyo, Japan) and water *ad libitum*. Light was for 12 h a day starting at 08.00 h. The rats were killed by cervical dislocation and their epididymal adipose tissue was quickly removed. Fat cells were isolated from the adipose tissue by the method of Rodbell.<sup>5</sup>

#### Measurement of noradrenaline-induced lipolysis in fat cells

An aliquot of the fat cell fraction (50  $\mu\text{l}$  packed volume) was incubated for 1 h at 37°C in 200  $\mu\text{l}$  of Hanks balanced solution (pH 7.4) supplemented with 2.5% BSA, noradrenaline (25  $\mu\text{l}$ , final concentration: 0.05  $\mu\text{g}/\text{ml}$ ) and the indicated amounts of test compounds (25  $\mu\text{l}$ ). The release of free fatty acid (FFA) was measured as described previously.<sup>6</sup> Briefly, the incubation mixture (250  $\mu\text{l}$ ) was mixed with 3 ml of chloroform-n-heptane (1:1, v/v) containing 2% methanol and extracted by shaking the tube horizontally for 10 min in a shaker. The mixture was centrifuged at 2000 g at 25°C for 5 min, and the upper aqueous phase was removed by suction. Copper reagent (1 ml) was then added to the lower organic phase. Then the tube was shaken for 10 min, the mixture was centrifuged at 2000 g at 25°C for 10 min, and 0.5 ml of the upper organic phase (which contained the copper salts of the extracted fatty acid) was treated with 0.5 ml of 0.1% (w/v) bathcurproine in chloroform containing 0.05% (w/v) 3-(2)-tert-

butyl-4-hydroxyanisole. Its absorbance was then measured at 480 nm. The glycerol released was measured by the method of Warnick.<sup>7</sup> Lipolysis was expressed as  $\mu\text{mol}$  FFA released per ml packed fat cells per 1 h, or  $\mu\text{mol}$  glycerol release per ml packed fat cells per 1 h.

#### Preparation of hormone-sensitive lipase (HSL) solution

Rat epididymal adipose tissue (0.7 g) was cut into small pieces with scissors, and homogenized in 1 ml of Hanks balanced solution (pH 7.4) in a Potter-Elvehjem homogenizer (Iuchi-Seiei-Do, Co., Tokyo, Japan) by five strokes of a Teflon pestle. The homogenate was centrifuged for 15 min at 2500 g at 10°C, and the supernatant was applied to a heparin-Sepharose column (5 × 20 mm), equilibrated with Hanks balanced solution, to remove lipoprotein lipase. The unadsorbed fraction was used as the HSL solution. HSL activity in this fraction was not reduced by 1 M NaCl or antiserum to bovine lipoprotein lipase, indicating that lipolytic activity due to lipoprotein lipase was minimal in the preparation.

#### Measurement of HSL activity in fat cells

Unlabelled triolein (50 mg) and [<sup>3</sup>H] triolein (220  $\mu\text{Ci}$  = 8.14 MBq) were suspended in 3.75 ml of 5% (w/v) gum arabic solution and sonicated for 5 min. The assay system contained the following components in a total volume of 100  $\mu\text{l}$ : 50  $\mu\text{l}$  HSL solution, 0.14  $\mu\text{mol}$  triolein, 0.45 mg gum arabic, 1.43 mg BSA, 8  $\mu\text{mol}$  KCl, 2 mmol NaCl and 4.5  $\mu\text{mol}$  *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES). The incubation was carried out for 1 h at 37°C at pH 6.8, and released [<sup>3</sup>H] oleic acid was measured by the method of Belfrage and Vaughan.<sup>8</sup> Lipase activity was expressed as nmol of [<sup>3</sup>H] oleic acid released per ml packed fat cells per 1 h.

#### Preparation of endogenous lipid droplets

Isolated fat cells were obtained from epididymal adipose tissues by the method of Rodbell.<sup>5</sup> A 1 ml packed volume of cells was suspended in 4 ml of 5 mM Tris-HCl buffer (pH 7.4). The suspension was mixed by slowly inverting the centrifuge three times and then centrifuged at 200 g at 25°C for 3 min. The fat layer was mixed with 4 ml of 5 mM Tris-HCl buffer (pH 7.4) containing 0.025% Triton X-100 by slowly swinging the tube three times, and the mixture was centrifuged at 200 g at 25°C for 3 min. The fat layer was washed once with Hanks balanced solution (pH 7.4) and suspended in the same buffer. This suspension was named 'endogenous lipid droplets'<sup>9</sup> and used in this study.

#### Measurement of noradrenaline-induced lipolysis in a cell-free system consisting of HSL and endogenous lipid droplets

An aliquot of endogenous lipid droplets (25  $\mu\text{l}$  packed volume) was incubated for 1 h at 37°C in 100 ml of

Hanks balanced solution (pH 7.4) supplemented with 2.5% BSA, noradrenaline (25  $\mu\text{l}$ , final concentration: 0.01  $\mu\text{g}/\text{ml}$ ), HSL fraction (100  $\mu\text{l}$ ) and the indicated amounts of test compounds (25  $\mu\text{l}$ ). Another experiment was performed as follows: A 25  $\mu\text{l}$  aliquot of endogenous lipid droplets was mixed with 125  $\mu\text{l}$  of Hanks balanced solution containing 2.5% BSA and 1.017 mg of gum arabic, and then the mixture was sonicated for 5 min. The sonicate (75  $\mu\text{l}$ ) was incubated with the HSL fraction (100  $\mu\text{l}$ ), Hanks balanced solution containing 2.5% BSA (50  $\mu\text{l}$ ), noradrenaline (25  $\mu\text{l}$ , final concentration: 0.05  $\mu\text{g}/\text{ml}$ ) and the indicated amounts of test compounds (25  $\mu\text{l}$ ) for 1 h at 37°C. The release of FFA was measured by the method described above. Lipolysis was expressed as  $\mu\text{mol}$  FFA released per ml packed lipid droplets per 1 h.

#### Measurement of pancreatic lipase activity

Lipase activity was determined by measuring the rate of release of oleic acid from triolein. A suspension of 90  $\mu\text{mol}$  triolein, 12.6  $\mu\text{mol}$  lecithin, and 9.45  $\mu\text{mol}$  taurocholic acid in 9 ml of 0.1 M *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), pH 7.0, containing 0.1 M NaCl was sonicated for 5 min. The assay system contained the following components in a total volume of 200  $\mu\text{l}$ : 50  $\mu\text{l}$  pancreatic lipase, 50  $\mu\text{l}$  test compound solution, 0.5  $\mu\text{mol}$  triolein, 0.053  $\mu\text{mol}$  taurocholic acid, 0.07  $\mu\text{mol}$  lecithin, 20  $\mu\text{mol}$  TES, and 20  $\mu\text{mol}$  NaCl. Incubation was carried out at pH 7.0 and 37°C for 30 min. The released oleic acid was determined by the method described above.

#### Statistical analysis

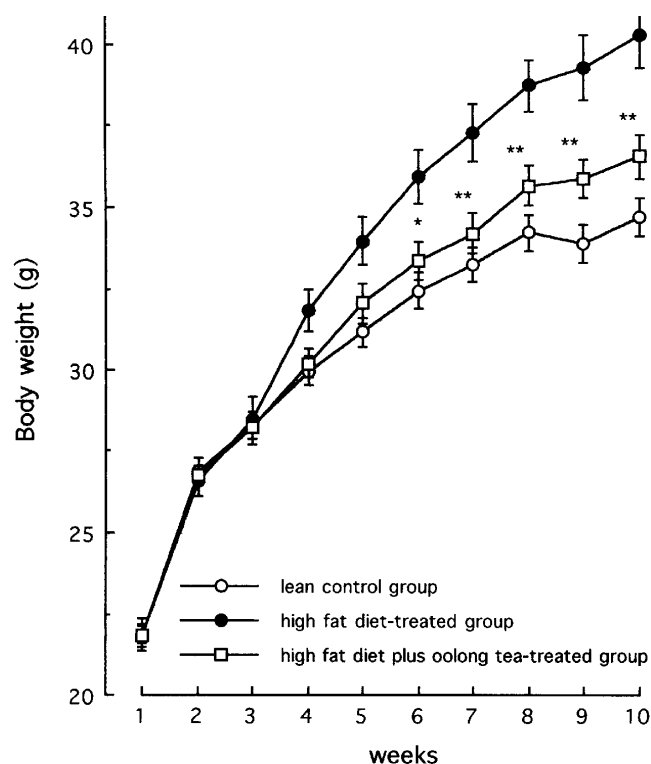
Results are expressed as means  $\pm$  standard error (s.e.m.) for each experiment. Statistical analysis was performed by Student's *t*-test to determine significance.

## Results

#### Body, parametrial adipose and liver weights, liver triglyceride and food consumption

Figure 1 shows the changes in body weights of the groups during the experiment. Feeding a high-fat diet containing 40% beef tallow for 10 weeks produced significant increases in body weight and parametrial white adipose tissue (WAT) weight as compared to laboratory chow-fed mice (normal group). Furthermore, the high-fat diet also induced fatty liver, with the accumulation of triglyceride when compared to the normal group (Table 1). Feeding a high-fat diet containing 5% oolong tea powder reduced the increases in body weight and final parametrial adipose tissue, and the accumulation of liver triglyceride as compared to high-fat diet-fed group (Figure 1 and

Table 1). The rate of reduction in body weight corresponded with that in parametrial adipose tissue weight. The mean food consumption per week per mouse during the whole experimental period was significantly ( $P < 0.05$ ) different between the laboratory chow and high-fat diet groups, being  $98.5 \pm 2.13$  kcal in the laboratory chow group and  $147.8 \pm 7.8$  kcal in the high-fat diet group. There was no significant difference in food consumption between the high-fat diet group and high-fat plus oolong tea diet ( $161.5 \pm 8.9$  kcal per mouse per week). These results suggested that the prevention of high-fat diet-induced obesity by oolong tea may be due to a decrease in intestinal absorption of lipid and/or acceleration of lipolysis in adipose tissue. Therefore, we attempted to isolate a lipolytic factor from oolong tea using a noradrenaline-induced lipolysis assay and an inhibitory assay for pancreatic lipase.



**Figure 1** Effects of oolong tea on body weight in mice fed a high-fat diet for 10 weeks. Values are expressed as mean  $\pm$  s.e.m. of 18 mice in each group. Significantly different from the high-fat diet-treated group; \*  $P < 0.05$ , \*\*  $P < 0.01$ .

**Table 1** Effects of oolong tea on parametrial adipose tissue weight, liver weight and hepatic triglyceride in mice fed a high-fat diet for 10 weeks

Group	Parametrial adipose tissue (g) Mean $\pm$ s.e.m.	Liver (g) Mean $\pm$ s.e.m.	Hepatic triglyceride (mg/g) Mean $\pm$ s.e.m.
Lean control	0.886 $\pm$ 0.10*	1.271 $\pm$ 0.03*	63.27 $\pm$ 5.39*
High-fat diet-treated	1.392 $\pm$ 0.1	2.243 $\pm$ 0.1	116.5 $\pm$ 6.7
High-fat diet plus 5% oolong tea powder-treated	0.674 $\pm$ 0.1*	2.173 $\pm$ 0.1	49.82 $\pm$ 3.7*

Results are expressed as mean  $\pm$  s.e.m. of 18 mice. Significantly different from high-fat diet-treated group; \*  $P < 0.01$ .

### Isolation of noradrenaline-augmenting lipolytic substance

As shown in Table 2, the water extract and its fractions corresponding to molecular weight (MW) below 1 kDa and 1–3 kDa enhanced noradrenaline-induced lipolysis at a concentration of 1000  $\mu$ g/ml. Furthermore, fraction B, which is separated from below 1 kDa MW by reverse-phase HPLC, enhanced noradrenaline-induced lipolysis at a concentration of 1000  $\mu$ g/ml (Table 2). An active substance isolated from fraction B was identified as caffeine by direct spectrographic comparison with an authentic sample. It is confirmed that caffeine is not contained in the 1–3 kDa MW fraction by the HPLC analysis. The isolation of lipolytic substances from the 1–3 kDa MW fraction are now in progress.

### Lipolytic activity of caffeine in fat cells

We found that caffeine enhanced noradrenaline-induced lipolysis in fat cells at low concentrations (10  $\mu$ M and 50  $\mu$ M), whereas it failed to stimulate lipolysis at the same concentration range in the absence of noradrenaline (Figure 2). It was found that both noradrenaline and caffeine modulated lipolysis without a concomitant increase in HSL activity (data not shown).

### Lipolytic activity of caffeine in a cell-free system

A negligible amount of free fatty acids was released in the cell-free system, consisting of lipid droplets and HSL, and noradrenaline-induced lipolysis in the system. As shown in Figure 3, caffeine enhanced the noradrenaline-induced lipolysis at the concentrations of 10–1000  $\mu$ M, in a dose-dependent manner. When the lipid droplets were replaced by sonicated ones in the cell-free system, the release of free fatty acid increased from 0.112–14.6  $\mu$ mol/ml packed lipid droplets per 1 h in the absence of noradrenaline, and the addition of the hormone and/or caffeine did not affect lipolysis (Table 3).

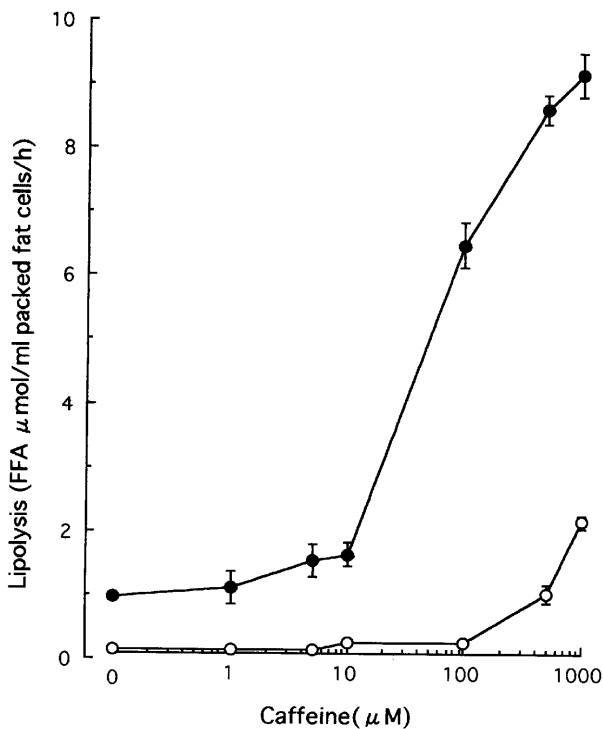
### Pancreatic lipase activity of oolong tea and its components

It has been clinically reported that a pancreatic lipase inhibitor orlistat (Ro 18-0647) prevented obesity and hyperlipidaemia after treatment for 12 weeks through inhibition of fat absorption.<sup>10–13</sup> In the present study, we found that the water extract of oolong tea inhibited pancreatic lipase at the concentrations of 500–

**Table 2** Effects of the water extract of oolong tea, < 1 kDa, 1–3 kDa, 3–10 kDa, 10–30 kDa, 30–100 kDa and > 100 kDa molecular weight (MW) fractions (fractionation by Diaflo Ultrafilter membrane) and various fractions (fraction A, B, C, D and E) (fractionation from the below 1 kDa MW fraction by HPLC) on noradrenaline-induced lipolysis in isolated fat cells from rats

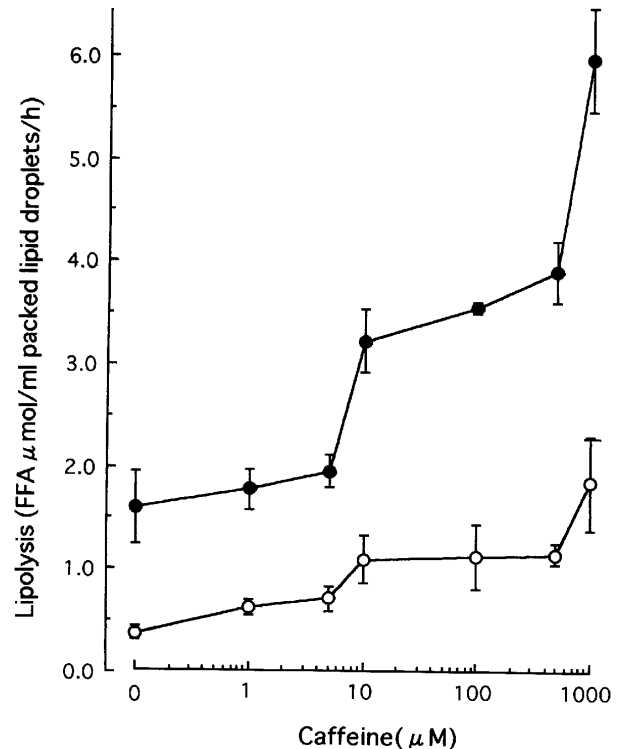
Additions (/ml reaction mixture)	Lipolysis (FFA $\mu\text{mol/ml}$ packed fat cells/h) Mean $\pm$ s.e.m.	% of Control
None	0 $\pm$ 0	–
Noradrenaline (0.05 $\mu\text{g}$ )	6.03 $\pm$ 0.43	100
Noradrenaline + water extract (100 $\mu\text{g}$ )	6.49 $\pm$ 0.20	107.6
(500 $\mu\text{g}$ )	8.53 $\pm$ 0.50	141.5
(1000 $\mu\text{g}$ )	10.52 $\pm$ 0.22	174.5
None	0 $\pm$ 0	–
Noradrenaline (0.05 $\mu\text{g}$ )	4.81 $\pm$ 0.36	100
Noradrenaline + < 1 kDa MW (1000 $\mu\text{g}$ )	7.69 $\pm$ 0.11	159.9
+1–3 kDa MW (1000 $\mu\text{g}$ )	7.25 $\pm$ 0.24	150.7
+3–10 kDa MW (1000 $\mu\text{g}$ )	6.34 $\pm$ 0.54	131.8
+10–30 kDa MW (1000 $\mu\text{g}$ )	4.97 $\pm$ 0.44	103.3
+30–100 kDa MW (1000 $\mu\text{g}$ )	6.37 $\pm$ 0.64	132.4
+> 100 kDa MW (1000 $\mu\text{g}$ )	5.69 $\pm$ 0.34	118.3
None	0 $\pm$ 0	–
Noradrenaline (0.05 $\mu\text{g}$ )	5.70 $\pm$ 0.37	100
Noradrenaline + fraction A (1000 $\mu\text{g}$ )	2.02 $\pm$ 0.03	35.4
+fraction B (1000 $\mu\text{g}$ )	12.02 $\pm$ 0.40	210.9
+fraction C (1000 $\mu\text{g}$ )	5.80 $\pm$ 0.16	101.8
+fraction D (1000 $\mu\text{g}$ )	1.61 $\pm$ 0.13	28.2
+fraction E (1000 $\mu\text{g}$ )	4.08 $\pm$ 0.26	71.6

Results are expressed as mean  $\pm$  s.e.m. of three experiments.



**Figure 2** Effects of caffeine on lipolysis in the presence or absence of noradrenaline in fat cells.  $\circ$ , caffeine alone;  $\bullet$ , noradrenaline (0.01  $\mu\text{g/ml}$ ) plus caffeine. Values are expressed as mean  $\pm$  s.e.m. of four experiments. FFA = free fatty acid.

2000  $\mu\text{g/ml}$ , in dose-dependent manner (Figure 4). Although caffeine was identified as an activator of noradrenaline-induced lipolysis, it failed to inhibit pancreatic lipase activity (data not shown). Tea tannins such as epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate also had no effect



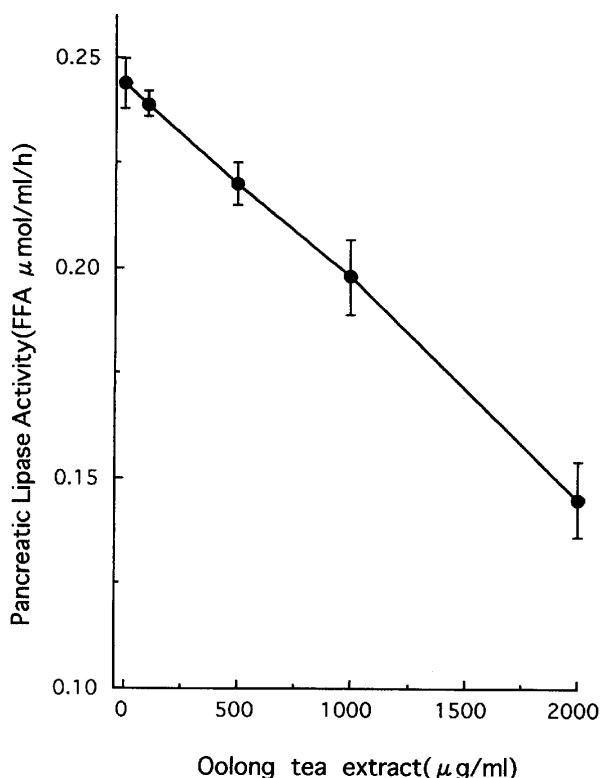
**Figure 3** Effects of caffeine lipolysis in a cell-free system consisting of intact lipid droplets and hormone-sensitive lipase (HSL) in the presence or absence of noradrenaline.  $\circ$ , caffeine alone;  $\bullet$ , noradrenaline (0.01  $\mu\text{g/ml}$ ) plus caffeine. Values are expressed as mean  $\pm$  s.e.m. of four experiments. FFA = free fatty acid.

on the pancreatic lipase activity (data not shown). These results suggest that the inhibition of pancreatic lipase activity may be caused by tea saponin fractions in oolong tea different from caffeine and tannins.

**Table 3** Effects of caffeine on lipolysis in a cell-free system consisting of sonicated lipid droplets and hormone-sensitive lipase (HSL) in the presence or absence of noradrenaline

Additions	Lipolysis (FFA $\mu\text{mol/ml}$ packed sonicated lipid droplets/h)	
	Mean $\pm$ s.e.m.	% of Control
None	14.6 $\pm$ 0.11	100.0
Noradrenaline (0.05 $\mu\text{g/ml}$ )	14.6 $\pm$ 0.13	100.0
Noradrenaline + caffeine (1000 $\mu\text{M}$ )	14.7 $\pm$ 0.23	100.7
Caffeine (1000 $\mu\text{M}$ )	14.7 $\pm$ 0.21	100.7

Results are expressed as mean  $\pm$  s.e.m. of four experiments.



**Figure 4** Effects of the water extract of oolong tea on pancreatic lipase activity. Values are expressed as mean  $\pm$  s.e.m. of four experiments. FFA = free fatty acid.

Experiments are now in progress to identify this inhibitory substance.

## Discussion

In the present study, we found that the oral administration for 10 weeks of a high-fat diet containing 40% beef tallow caused obesity with increases in parametrial WAT weight. Furthermore, a high fat diet containing 40% beef tallow also induced fatty liver with the accumulation of hepatic triglycerides. The present investigation found that oolong tea prevented the increases in body weights and parametrial adipose tissue in mice fed a high-fat diet containing 40% beef tallow for 10 weeks. Hepatic triglyceride content was also reduced by

the administration of oolong tea, compared to the high-fat diet-treated group, while the liver weight was nearly the same in the high-fat diet and high-fat diet plus oolong tea groups. It was reported that the anti-hyperlipidaemic drug clofibrate caused liver hypertrophy in rats and mice.<sup>14</sup> Caffeine caused the liver hypertrophy.<sup>15</sup> It seems likely that the anti-obesity and anti-fatty liver actions of oolong tea may also cause the liver hypertrophy in a similar manner to the hypolipidaemic drug clofibrate. In this study, high-fat diet and high-fat diet containing 5% oolong tea did not cause liver injury, in that there was no elevation of serum GOT and GPT (data not shown). Further study is needed to clarify the mechanisms of liver hypertrophy due to oolong tea. These results suggest that oolong tea might exert its anti-obesity action through inhibition of intestinal absorption of dietary fat, acceleration of lipolysis in adipose tissue and other mechanisms. We therefore attempted to isolate active substances from oolong tea which may be related to its anti-obesity action. At first, caffeine was isolated from oolong tea as a potential activator of noradrenaline-induced lipolysis in fat cells. It has been reported that caffeine ingestion caused the elevation of the metabolic rate and fatty acid availability, through lipolysis in fat cells and the release of catecholamines.<sup>16–20</sup>

In the present study, we found that caffeine isolated from oolong tea enhanced noradrenaline-induced lipolysis in fat cells at relatively low concentrations, 1–10  $\mu\text{M}$ . The biochemical mechanisms of xanthine derivatives such as caffeine and theophylline have been attributed to the adenylylcyclase–cyclic AMP (cAMP) phosphodiesterase cycle.<sup>21</sup> The cAMP-dependent protein kinase A, in turn activates HSL and the activated HSL catalyses the hydrolysis of triglyceride in fat cells.<sup>22,23</sup> However, Okuda *et al*<sup>24</sup> found that cAMP-dependent activation of HSL stimulated lipolysis of [<sup>3</sup>H]triolein emulsified with gum arabic, but not of endogenous lipid droplets prepared from fat cells. They prepared the endogenous lipid droplets from fat cells by mild procedures, including hypotonic shock Triton X-100 treatment and brief incubation in a buffer. Approximately 95% of the protein present in the cells was removed from the droplets, which consisted mainly of triglyceride, with phosphatidylcholine, phosphatidylethanolamine,

cholesterol, carbohydrate, and protein, as minor constituents.<sup>25</sup> The endogenous lipid droplets were found to show lipolysis in response to catecholamines, theophylline and *p*-aminophenol.<sup>26–28</sup> Furthermore, Okuda *et al*<sup>29,30</sup> demonstrated that sonication of lipid droplets caused the loss of responsiveness to catecholamine and  $\beta$ -blockers in a cell-free system consisting of the lipid droplets and HSL. Furthermore, noradrenaline stimulated lipolysis in the cell-free system, whereas lipid droplets did not exhibit specific binding of (–)-[<sup>3</sup>H]dihydroalprenolol, which was used as a  $\beta$ -adrenergic receptor assay. Based on these results, Okuda and colleagues<sup>6,30</sup> suggested that specific (–)-[<sup>3</sup>H]dihydroalprenolol binding sites in rat fat cells might not participate in the catecholamine-induced lipolysis, and a different receptor concerned with lipolysis may exist with close relation to the lipid droplets in the fat cells through mechanisms other than the cAMP cascade, and that lipid droplets may be a limiting factor for lipolysis in fat cells. Pretreatment of the endogenous lipid droplets with phospholipase C greatly reduced catecholamine-induced lipolysis, whereas pretreatment with phospholipase D did not affect the lipolytic activities. These facts suggest that phosphate groups in the phospholipids of the endogenous lipid droplets may play an important role in the lipolytic actions of catecholamines. In the present experiments, caffeine isolated from oolong tea enhanced noradrenaline-induced lipolysis in both fat cells and a cell-free system consisting of HSL and endogenous lipid droplets. Moreover, we found that the sonication of lipid droplets caused the loss of the response of noradrenaline and caffeine in a cell free-system consisting of the sonicated lipid droplets and HSL. This fact suggests that noradrenaline and caffeine might not exert effects in HSL, directly. We previously reported that adrenaline, dibutyryl cAMP, theophylline and *p*-aminophenol, which have a positive charge and a hydrophobic area in their structural skeletons, have a lipolytic effect on endogenous lipid droplets from rat fat cells.<sup>26</sup> On the basis of the above results, we postulated that catecholamine, dibutyryl cAMP, or theophylline increased the hydrophobicity on the surface of lipid droplets by binding to the phosphate groups of phospholipids, subsequently inducing association between the lipase and the triglyceride portion of lipid droplets, thus eliciting lipolysis. Caffeine also possesses both a positive charge and hydrophobic area, like adrenaline, dibutyryl cAMP and theophylline. Therefore, it is suggested that the mechanism of the lipolytic actions of caffeine might also be due to binding to the phosphate groups of phospholipids, and subsequent interaction between the lipase and triglyceride portion of lipid droplets.

In addition, it was found that a water extract of oolong tea inhibited pancreatic lipase. Caffeine and tea tannins such as epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate had no effect on the pancreatic lipase activity. Therefore,

oolong tea may contain a substance different from caffeine and tannins, that inhibits intestinal absorption of dietary fat.

## Conclusion

It was demonstrated that the anti-obesity effects of oolong tea in high-fat diet-treated mice might be partly due to the enhancement by caffeine of noradrenaline-induced lipolysis in adipose tissue and the inhibitory actions of some other substance in oolong tea on pancreatic lipase activity. The results suggest that oolong tea may be an effective crude drug for the treatment of obesity and fat liver caused by a high-fat diet.

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