Anti-Obesity Effects of a Mixture of Thiamin, Arginine, Caffeine, and Citric Acid in Non-Insulin Dependent Diabetic KK Mice

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Summary Anti-obesity effects of a mixture of thiamin, arginine, caffeine, and citric acid (TACC) were investigated in non-insulin dependent diabetic KK mice. Feeding of either arginine or caffeine significantly suppressed an increase in hepatic lipid contents in fasted-refed KK mice. In addition, each component admixed with a low-calorie diet effectively reduced adipose tissue weight in KK mice previously fed a high-calorie diet. The decrease in adipose tissue weight was greater with a mixture of arginine and caffeine, and much greater with TACC than with arginine or caffeine alone. Moreover, plasma insulin concentration was significantly lower in mice fed TACC than in control mice. The anti-obesity effects of TACC were also shown when it was supplemented with a tea beverage. Adipose tissue weight, hepatic triglyceride contents, and plasma insulin concentration were significantly lower in mice given TACC-supplemented tea than in control mice. These results suggest that TACC is effective in reducing adipose tissue mass as well as improving disorders in lipid metabolism.

Key Words obesity, mice, arginine, caffeine, lipid metabolism

Obesity has become prevalent in industrialized countries as a result of changes in lifestyle, especially in eating habits. It is known to be associated with a number of serious medical complications such as non-insulin dependent diabetes mellitus (NIDDM), hyperlipidemia, hypertension, and cardiovascular disease (1, 2). Therefore, prevention and treatment of obesity are relevant to promoting better health. Various food components have been reported to influence lipid metabolism and to be effective in prevention and treatment of obesity (3–8). Among these, we focused on thiamin, arginine, caffeine, and citric acid to develop an anti-obesity agent by composing a mixture of these substances (TACC).

Thiamin (vitamin B1) is essential for energy metabolism. Deficiency of vitamin B1, which is liable to occur during restricted dietary intake, induces a decrease in metabolic rate (9). Its requirement is parallel to energy expenditure. Thus, the addition of thiamin to our anti-obesity agent was thought meaningful to support elevated energy expenditure and to be effective in prevention and treatment of obesity (3–8). Among these, we focused on thiamin, arginine, caffeine, and citric acid to develop an anti-obesity agent by composing a mixture of these substances (TACC).

Thiamin (vitamin B1) is essential for energy metabolism. Deficiency of vitamin B1, which is liable to occur during restricted dietary intake, induces a decrease in metabolic rate (9). Its requirement is parallel to energy expenditure. Thus, the addition of thiamin to our anti-obesity agent was thought meaningful to support elevated energy expenditure, which is expected to be induced by the components present, arginine and caffeine. Several studies have shown that the intake of arginine stimulates the secretion of glucagon (10, 11), which directly enhances the lipolysis of human adipose tissue (12). In fact, physiologically as well as pharmacologically higher levels of blood glucagon have been shown to be associated with increases in free fatty acid (FFA) and glycerol in the blood (13–15).

Caffeine is a well-characterized lipolytic substance. It promotes the lipolysis of adipose tissue through the inhibition of phosphodiesterase activity, which decreases the cellular level of cyclic adenosine monophosphate (16, 17). Moreover, in vivo treatment with caffeine decreases body weight in association with a reduction in fat mass through an increase in energy expenditure in rodents (18, 19). It is generally accepted that citric acid is a regulator of glycolytic flux, having an inhibitory effect on the activity of phosphofructokinase (20). Several studies have shown that there is a relationship between elevated citric acid and reduced glycolysis/glycogenolysis (21, 22). Thus, citric acid has a potential to increase fatty acid utilization through sparing liver and muscle glycogen.

In this study, we investigated the anti-obesity actions of TACC. First, anti-lipogenic and adipose tissue-reducing effects of either arginine or caffeine were examined in non-insulin dependent diabetic KK mice. Then, those effects of individual agents were compared with the effects of a mixture of the two. Finally, we examined the effects of TACC combined with a low-calorie diet on adipose tissue weight and obesity related disorders in KK mice previously fed a high-calorie diet.

MATERIALS AND METHODS

Animals. All animal experiments in this study were approved by the ethical committee for the animal experiments of the Pharmaceutical Research Division.
Anti-Obesity Effects of TACC in NIDDM Mice

Takeda Chemical Industries, and carried out in a facility of the division. Male, 5- to 7-wk-old, KK/Ta mice were purchased from CLEA Japan (Tokyo, Japan). They were fed a laboratory chow diet (CE-2; CLEA Japan) ad libitum for 7 d for acclimation before experiments began. The mice were allowed free access to food and tap water and housed in individual cages. The animal room was maintained at 23±1°C, 55±5% humidity and 12-h light/dark cycle.

TACC. Dibenzoyl thiamin hydrochloride (DBT-HCl), a thiamin derivative used as a food additive, was purchased from Takeda Chemical Industries (Osaka, Japan). Arginine was purchased from Kyowa Hakko Kogyo Co. (Tokyo, Japan). Caffeine, extracted from coffee beans (more than 98.5% purity), was purchased from Shiratori Pharmaceutical Co. (Chiba, Japan). Citric acid was purchased from Archer Daniels Midland (Illinois, USA).

Materials and basal diets. The compositions of a high-carbohydrate diet (HCHO, 73.4% carbohydrate), a high-calorie diet (HCAL, 4.7 kcal/g) and a low-calorie diet (LCAL, 3.1 kcal/g) used in this study are shown in Table 1. Casein, corn oil, cellulose powder, Harper mineral mixture (23), and Harper vitamin mixture (23) were purchased from Oriental Yeast Co. (Tokyo, Japan). Cornstarch was purchased from Japan Cornstarch (Aichi, Japan). Choline bitartrate was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Drinking fluid. The source materials used for preparation of a blend-tea are shown in Table 2. These materials were obtained from Makise & Co. (Osaka, Japan). After extracting 100 g of a mixture of the materials with 2.8 L of deionized water at 90°C for 5 min, the extract was diluted with deionized water up to 10 L to obtain a blend-tea. The blend-tea was supplemented with or without TACC and pasteurized at 115°C for 20 min. The quantities of TACC (a low and a high dose), added and measured, in drinking fluid are shown in Table 3. Portions of added DBT-HCl and arginine were decomposed during heat treatment.

Effects of arginine and caffeine on lipogenesis in the liver. KK mice (8-wk-old) were fasted for 48 h and then divided into three groups (n=6). The control group was re-fed HCHO and the experimental groups were re-fed HCHO supplemented with either arginine (900 mg/100 g diet) or caffeine (37.5 mg/100 g diet) for 72 h. After refeeding, livers were removed and stored at −20°C for measurement of lipid contents.

Effects of main TACC components (arginine and caffeine) and TACC on obesity. Obesity was induced in KK mice (6-wk-old) by feeding HCAL for 2 wk and then the mice were used for experiments 1–3. In the first experiment, the mice were divided into four dietary treatment groups (n=6). The control group was fed LCAL, and the experimental groups were fed LCAL supplemented with either arginine (600 mg/100 g diet) or caffeine (25 mg/100 g diet) or both for 2 wk. Body weight and calorie intake were measured every 2 to 3 d. After feeding LCAL, subcutaneous.

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Table 1. Composition of experimental diets (%).

| Ingredient               | Diet 1 | HCHO | HCAL | LCAL |
|--------------------------|--------|------|------|------|
| Casein                   | 18     | 18   | 18   |
| Cornstarch               | 73.4   | 53.4 | 53.4 |
| Corn oil                 | —      | 20   | 2.5  |
| Cellulose powder         | 2.5    | 2.5  | 20   |
| Harper mineral mixture   | 5      | 5    | 5    |
| Harper vitamin mixture   | 1      | 1    | 1    |
| Choline bitartrate       | 0.1    | 0.1  | 0.1  |

1 HCHO, high-carbohydrate diet; HCAL, high-calorie diet; LCAL, low-calorie diet.
2 Ref. 23.

Table 2. Mixing rate of source materials in blend-tea.

| Source materials          | wt/wt % |
|---------------------------|---------|
| Green tea                 | 33      |
| Job's tears               | 29      |
| Barley                    | 16      |
| Brown rice                | 12      |
| Oolong tea                | 5       |
| Dokudami                  | 4       |
| Tu-chung tea              | 1       |

Table 3. Contents of TACC components in the drink.

| Blended tea                | TACC-supplemented tea |
|----------------------------|-----------------------|
|                            | Low dose  | High dose  |
|                            | Added     | Measured  | Added     | Measured  |
|                            | (mg/100 g of tea) |            | (mg/100 g of tea) |            |
| DBT-HCl1                   | 0         | —         | 0.29      | 0.16      |
| Arginine                   | 0         | —         | 118       | 109       |
| Caffeine                   | 0         | 4         | 5         | 9         |
| Citric acid                | 0         | —         | 59        | 59        |

1 DBT-HCl, dibenzoyl thiamin hydrochloride.
—: not detected.
perirenal/retroperitoneal, epididymal and mesenteric adipose tissues were removed and weighed.

In the second experiment, the mice were divided into three dietary treatment groups (n=5 or 6). The control group was fed LCAL, and the experimental groups were fed LCAL supplemented with either a mixture of arginine and caffeine (600 mg and 25 mg/100 g diet, respectively) or TACC (1 mg, 600 mg, 25 mg and 270 mg/100 g diet for DBT-HCl, arginine, caffeine and citric acid, respectively) for 2 wk. Body weight, calorie intake, and adipose tissue weight were measured as described above. Before and after feeding LCAL, blood was sampled from the orbital sinus for biochemical analysis. Livers were removed for analysis of hepatic lipids after LCAL feeding.

In the third experiment, the mice were divided into four dietary treatment groups (n=6 or 7). All groups were fed LCAL. The control group was given a blend tea, the experimental groups were given a low dose or a high dose of TACC supplemented tea, and the normal group was given tap water for 2 wk. Body weight, calorie intake, and adipose tissue weight were measured as described above. Biochemical analysis of blood and analysis of hepatic lipids were carried out as described above.

Biochemical analysis. Glucose, triglyceride, cholesterol and FFA concentrations in the plasma were enzymatically determined by a Hitachi Automatic Analyzer 7070 (Hitachi Ltd., Ibaraki, Japan). Plasma insulin was measured by double-sandwiched ELISA using a commercially available kit (GLAZYME Insulin-EIA TEST, Wako).

Hepatic lipid analysis. Hepatic lipid was extracted according to the method of Folch et al. (24). Triglyceride, phospholipid and cholesterol contents were determined using commercially available kits (Triglyceride-Test Wako, Phospholipid-Test Wako and Cholesterol C-Test Wako, Wako).

Statistical analysis. Data are expressed as means±SD. All statistical analyses were performed using Dr. SPSS software version 8.0J (SPSS Japan Inc., Tokyo, Japan). Differences between control and treated groups were analyzed by Dunnnett's test. As plasma insulin values did not follow normal distribution, according to Shapiro-Wilk test, Mann-Whitney test was used to compare control and treated groups. Significance level was set at p<0.05.

RESULTS

Effects of arginine and caffeine on lipogenesis in the liver
To clarify the individual effects of arginine and caffeine, main components of TACC, on lipid metabolism, we first examined the anti-lipogenic actions of each of these two components in a fasted-refed model. KK mice fasted for 48 h and refed HCHO for 72 h showed markedly high contents of hepatic triglyceride (38.7±8.7 mg/g) in the control mice (Table 4). However, hepatic triglyceride contents were significantly lower in the mice given arginine (p=0.001) or caffeine (p<0.001) after the refeeding period (Table 4). Arginine and caffeine are considered to be effective in suppressing the synthesis of fatty acid in the liver. Hepatic phospholipid and cholesterol contents were also significantly lower in the mice given arginine (p=0.002 and p<0.001, respectively) or caffeine (p<0.001 and p<0.001, respectively) than in the control mice (Table 4). There were no differences in body weight change or calorie intake among the three groups (Table 4).

Effects of TACC on obesity and obesity-related disorders
We examined the effects of main TACC components and TACC on obesity by administering them individually (either arginine or caffeine) or in combination (either a mixture of arginine and caffeine or TACC) to KK mice. To better determine their anti-obesity actions, these supplements combined with LCAL were given to KK mice that had been previously made obese by HCAL. As shown in Table 5, there was no significant difference in the calorie intake among the groups of mice fed one of the following LCAL diets for 2 wk: 1, LCAL; 2, LCAL with arginine; 3, LCAL with caffeine; 4, LCAL with arginine and caffeine. Body weight gain and adipose tissue weight in the mice given arginine or caffeine were slightly lower than those in the control mice, but the differences were not statistically significant (Table 5). On the other hand, body weight gain was significantly

| Table 4. Effects of arginine and caffeine on hepatic lipid contents in fasted-refed mice. |
|---------------------------------------------------------------|
| **Dietary group** | **Control (HCHO)** | **HCHO+arginine** | **HCHO+caffeine** |
|---------------------------------------------------------------|
| Body weight (g) Fasted | 25.8±1.0 | 25.7±0.7 | 26.0±0.8 |
| Body weight (g) Refed | 29.6±1.4 | 29.9±1.5 | 29.3±0.6 |
| Calorie intake (kcal/72 h) | 51.5±10.5 | 49.9±2.6 | 45.7±11.9 |
| Relative liver weight (g/100 g b.w.) | 4.91±0.45 | 4.85±0.30 | 4.79±0.21 |
| Triglyceride (mg/g liver) | 38.7±8.7 | 19.0±8.4** | 12.3±6.6** |
| Phospholipid (mg/g liver) | 29.4±2.1 | 25.9±1.1** | 25.0±1.1** |
| Cholesterol (mg/g liver) | 3.61±0.12 | 2.91±0.29** | 2.43±0.31** |

1 HCHO, high-carbohydrate diet.

Values represent means±SD. n = 6. **p<0.01 vs. control.
Table 5. Effects of arginine and/or caffeine on adipose tissue weight in KK mice.

|                          | Control (LCAL)$^1$ | LCAL + arginine | LCAL + caffeine | LCAL + arginine & caffeine |
|--------------------------|--------------------|-----------------|-----------------|----------------------------|
| Body weight gain (% of initial) | 7.0±2.2            | 3.3±3.5        | 2.0±5.7$^1$     | 0.8±3.1$^*$                 |
| Calorie intake (kcal/d)   | 14.2±2.3           | 14.9±1.1       | 15.7±1.6       | 13.9±1.5                   |
| Adipose tissue weight (g/100 g b.w.) | 0.82±0.11        | 0.76±0.05      | 0.76±0.15      | 0.73±0.10                  |
| Subcutaneous             | 3.14±0.33          | 2.93±0.50      | 2.76±0.82      | 2.52±0.32                  |
| Epididymal               | 1.64±0.39          | 1.26±0.17      | 1.21±0.42$^1$  | 1.11±0.33$^*$              |
| Perirenal/retroperitoneal | 1.66±0.30          | 1.57±0.17      | 1.36±0.46      | 1.36±0.24                  |
| Mesenteric               | 7.26±1.07          | 6.51±0.75      | 6.08±1.82      | 5.72±0.90$^1$              |

$^1$ LCAL, low-calorie diet. Values represent means±SD, n=6. $^1$p<0.10 and $^*p<0.05$ vs. control.

Table 6. Effects of TACC on adipose tissue weight in KK mice.

|                          | Control (LCAL)$^1$ | LCAL + arginine & caffeine | LCAL + TACC |
|--------------------------|--------------------|----------------------------|-------------|
| Body weight gain (% of initial) | −2.4±3.5           | −6.7±6.1                   | −8.1±6.1    |
| Calorie intake (kcal/d)   | 15.7±1.0           | 14.6±1.2                   | 15.1±1.2    |
| Adipose tissue weight (g/100 g b.w.) | 0.80±0.07          | 0.73±0.08                  | 0.73±0.12   |
| Subcutaneous             | 3.23±0.47          | 2.94±0.67                  | 2.59±0.38$^1$ |
| Epididymal               | 1.39±0.21          | 1.27±0.31                  | 1.02±0.29$^1$ |
| Perirenal/retroperitoneal | 1.54±0.22          | 1.42±0.28                  | 1.22±0.24$^1$ |
| Mesenteric               | 6.96±0.93          | 6.35±1.29                  | 5.56±0.95$^1$ |

$^1$ LCAL, low-calorie diet. Values represent means±SD, n=5 or 6. $^1$p<0.10 vs. control.

lower ($p=0.029$) in the mice given a mixture of arginine and caffeine than in the control mice (Table 5). Perirenal/retroperitoneal adipose tissue weight was also significantly lower ($p=0.035$) and total adipose tissue weight tended to be lower ($p=0.097$) in the mice given a mixture of arginine and caffeine than in the control mice (Table 5). These results suggest that the effect of a mixture of arginine and caffeine on adipose tissue reduction is stronger than that of the individual components.

We next examined the effect of a combination of all the TACC components using the same experimental protocol as described above. After feeding HCAL for 2 wk, mice were fed LCAL supplemented with or without a mixture of arginine and caffeine or TACC for 2 wk. Body weight gain and all of the various adipose tissue weights were lower in the mice given either a mixture of arginine and caffeine or TACC than those in the control mice (Table 6). Only the mice given TACC, but not the mice given a mixture of arginine and caffeine, showed almost significantly lower weights in epididymal, perirenal/retroperitoneal, mesenteric, and total adipose tissues ($p=0.084, 0.061, 0.074$ and $0.067$ vs. control, respectively) than the control mice (Table 6).

Table 7. Effects of TACC on plasma components in KK mice.

|                          | Control (LCAL)$^1$ | LCAL + arginine & caffeine | LCAL + TACC |
|--------------------------|--------------------|----------------------------|-------------|
| Glucose (mg/dL)          | 288±43             | 257±26                     | 272±58      |
| Triglyceride (mg/dL)     | 244±61             | 164±67$^*$                 | 150±45$^*$  |
| Cholesterol (mg/dL)      | 112±10             | 102±26                     | 92±13       |
| Insulin (IU/dL)          | 290±222            | 266±460                    | 77±48$^*$   |

$^1$ LCAL, low-calorie diet. Values represent means±SD, n=5 or 6. $^1$p<0.10, $^*$p<0.05 vs. control.

To examine the effects of TACC in more detail, plasma components and hepatic lipid contents were measured in these mice. The level of plasma triglyceride was slightly lower in the mice given a mixture of arginine and caffeine, and was significantly lower ($p=0.026$) in mice given TACC than the level in the control mice (Table 7).

The level of plasma insulin, which usually increases
in non-insulin dependent diabetes mellitus, was significantly lower \((p=0.025)\) in the mice given TACC than the level in the control mice (Table 7). Hepatic triglyceride contents in the mice given TACC were lower than those in the control mice, but the difference was not statistically significant (Table 8). Hepatic cholesterol contents were significantly lower in the mice given either a mixture of arginine and caffeine \((p<0.001)\) or TACC \((p=0.002)\) than in the control mice (Table 8). The contents of hepatic phospholipid were not different among the groups (Table 8). These results suggest that the anti-obesity effects of TACC are stronger than those of a mixture of arginine and caffeine.

### Table 8. Effects of TACC on hepatic lipid contents in KK mice.

| Dietary group                  | Control (LCAL) 1 | LCAL + arginine & caffeine | LCAL + TACC |
|-------------------------------|------------------|---------------------------|-------------|
| Relative liver weight (g/100 g b.w.) | 3.87 ± 0.29   | 3.68 ± 0.25               | 3.73 ± 0.36 |
| Triglyceride (mg/g liver)     | 14.4 ± 5.3      | 13.5 ± 10.4               | 8.8 ± 5.2   |
| Phospholipid (mg/g liver)     | 29.2 ± 2.2      | 28.2 ± 1.0                | 28.3 ± 1.8  |
| Cholesterol (mg/g liver)      | 2.05 ± 0.20     | 1.41 ± 0.22**             | 1.59 ± 0.17** |

1 LCAL, low-calorie diet. Values represent means ± SD. \(n=5\) or 6. **\(p<0.01\) vs. control.

### Table 9. Effects of TACC-supplemented tea on adipose tissue weights in KK mice.

| Fluid group                  | Blended tea | TACC-supplemented tea | Tap water |
|-------------------------------|-------------|-----------------------|-----------|
|                                | Low dose    | High dose             |           |
| Body weight gain (% of initial)| 1.6 ± 2.8   | -2.0 ± 3.5            | -3.8 ± 3.6* |
| Calorie intake (kcal/d)       | 15.5 ± 2.5  | 15.6 ± 1.9            | 15.1 ± 1.5 |
| Fluid intake (g/d)            | 8.0 ± 1.9   | 7.9 ± 1.9             | 6.8 ± 1.2 |
| Adipose tissue weight (g/100 g b.w.) | 0.93 ± 0.06 | 0.84 ± 0.09           | 0.76 ± 0.13** |
| Subcutaneous                  | 3.36 ± 0.39 | 3.26 ± 0.40           | 2.84 ± 0.54† |
| Epididymal                    | 1.57 ± 0.29 | 1.49 ± 0.11           | 1.29 ± 0.32 |
| Perirenal/retroperitoneal     | 1.76 ± 0.24 | 1.62 ± 0.21           | 1.38 ± 0.25** |
| Mesenteric                    | 7.62 ± 0.91 | 7.21 ± 0.74           | 6.27 ± 1.22* |

Values represent means ± SD. \(n=6\) or 7. †\(p<0.10\), *\(p<0.05\) and **\(p<0.01\) vs. blended tea.

### Table 10. Effects of TACC-supplemented tea on plasma components in KK mice.

| Fluid group                  | Blended tea | TACC-supplemented tea | Tap water |
|-------------------------------|-------------|-----------------------|-----------|
|                                | Low dose    | High dose             |           |
| Glucose (mg/dL)               | 315 ± 35    | 310 ± 78              | 282 ± 33  |
| Triglyceride (mg/dL)          | 188 ± 78    | 165 ± 49              | 161 ± 60  |
| Cholesterol (mg/dL)           | 119 ± 18    | 110 ± 14              | 105 ± 18  |
| FFA (μEq/dL)                  | 665 ± 195   | 666 ± 129             | 617 ± 82  |
| Insulin (IU/dL)               | 271 ± 192   | 189 ± 351             | 124 ± 209* |

Values represent means ± SD. \(n=6\) or 7. *\(p<0.05\) vs. blended tea.
teric and total adipose tissue weights were also significantly lower (p=0.007, 0.009 or 0.030, respectively) in the mice given a high dose of TACC-supplemented tea than in the mice given blend-tea (Table 9).

The levels of plasma glucose, triglyceride, cholesterol, and FFA were not significantly different among the groups (Table 10). However, the plasma insulin level was significantly lower (p=0.032) in the mice given a high dose of TACC-supplemented tea than in the mice given the blend-tea (Table 10). Hepatic triglyceride contents in the mice given the blend-tea were at a considerably higher level. However, those in the mice given a low dose or a high dose of TACC-supplemented tea were significantly lower (p=0.045 or 0.009, respectively) than those in the control mice (Table 11). The contents of hepatic phospholipid and cholesterol were not different among the groups (Table 11).

**DISCUSSION**

In this study we have demonstrated the anti-obesity effects of TACC. Among the TACC components, arginine and caffeine were found to suppress lipogenesis in the liver and to enhance anti-obesity action induced by LCAL in KK mice. As shown in the mice given LCAL, the effect of TACC on adipose tissue reduction was stronger than that of a mixture of arginine and caffeine (Tables 6–8). Moreover, TACC-supplemented tea combined with LCAL effectively reduced adipose tissue weight and improved obesity-related disorders such as fatty liver and hyperinsulinemia in KK mice (Tables 9–11). Our results suggest that TACC is useful as a prophylactic and therapeutic agent against obesity-related disorders.

Refeeding a high-carbohydrate diet to fasted rats is known to activate the synthesis of lipogenic enzymes at the mRNA level as well as the protein level (25, 26). There have been, however, few reports that directly show the effect of arginine or caffeine on hepatic lipogenesis in fasted-reared animals. In the present experiments, we have clearly shown that either arginine or caffeine is effective for suppressing the development of fatty liver induced in KK mice by fasting-refeeding treatment (Table 4). Glucagon, the secretion of which is stimulated by caffeine (28, 29), are reported to inhibit lipogenesis in hepatocytes (30, 31). Therefore, the inhibitory effect of arginine or caffeine on hepatic lipogenesis is considered to be exerted through the secretory response of glucagon or catecholamines induced by arginine or caffeine.

The effect of a mixture of arginine and caffeine on adipose tissue reduction was greater than any of the individual components examined in this study (Table 5). We have other findings showing that a combination of arginine and caffeine synergistically promotes lipolysis in a 3T3-L1 adipocyte cell line (unpublished data), suggesting that the effect of a mixture of arginine and caffeine can be synergistically manifested even in vivo. The promotion of lipolysis in addition to the inhibition of lipogenesis can contribute to adipose tissue reduction. These actions of the two components seem to be exerted through their own specific mechanisms. Thus, a combination of arginine and caffeine is considered to show some additive effect on adipose tissue reduction. In fact, the present study shows the effect of TACC. Adipose tissue weights were lower in mice given TACC than in mice given a mixture of arginine and caffeine (Table 6). It has been suggested that increased utilization of intramuscular triglyceride and/or extramuscular FFA after caffeine ingestion may inhibit carbohydrate consumption at rest and at the beginning of exercise partly due to the elevation of citric acid concentration in the muscle (32). It is possible that exogenously increased levels of citric acid could help caffeine promote lipid utilization. Therefore, the effect of a mixture of caffeine and citric acid may contribute to strengthening those of TACC. Since we have not examined the detailed effect of a mixture of citric acid and other TACC components in this study, further studies are required to clarify the mechanism exhibited by a combination of TACC.

It is notable in this study that TACC improves hyperinsulinemia and fatty liver in KK mice that inherently have characteristics of glucose intolerance and insulin resistance (33). Insulin resistance can lead to diabetes, hyperinsulinemia, dyslipidemia, and hypertension (34). These metabolic disturbances constitute insulin resistance syndrome that has been shown to increase the risk of coronary artery disease (35, 36). It has been suggested that abdominal visceral obesity is a critical outcome of insulin resistance syndrome (37, 38). In

| Fluid group | Blended tea | TACC-supplemented tea | Tap water |
|-------------|-------------|-----------------------|-----------|
|             |             | Low dose | High dose |             |
| Relative liver weight (g/100 g b.w.) | 4.07±0.44 | 3.81±0.42 | 3.77±0.34 | 4.01±0.31 |
| Triglyceride (mg/g liver) | 23.9±9.8 | 14.1±3.9* | 10.9±6.6** | 29.5±6.7 |
| Phospholipid (mg/g liver) | 31.8±3.4 | 31.9±2.3 | 32.2±1.8 | 31.3±2.4 |
| Cholesterol (mg/g liver) | 1.99±0.24 | 1.85±0.13 | 1.81±0.10 | 2.15±0.29 |

Values represent means±SD. n=6 or 7. *p<0.05 and **p<0.01 vs. blended tea.
this paper, we have shown a significant reduction in mesenteric adipose tissue weight in mice given TACC-supplemented tea (Table 9). Therefore, TACC is expected to improve insulin resistance syndrome. In fact, the present results that mice given TACC showed a significantly lower level of plasma insulin suggest the effectiveness of TACC on reducing insulin resistance syndrome (Tables 7, 10). It has recently been shown that fatty liver is strongly associated with many features of insulin resistance syndrome (39). The present result that hepatic triglyceride contents were maintained at significantly lower levels in mice given TACC-supplemented tea as shown in Table 11 also suggests the useful influence of TACC for ameliorating insulin resistance syndrome.

It has been reported that the consumptions of green tea and oolong tea increase lipolysis, energy expenditure and fat oxidation, leading to the suppression of fat accumulation (7, 8, 40, 41). These effects are thought to be achieved by the caffeine and/or polyphenol in the teas (8, 40, 42). Since source materials used in the preparation of a blend-tea in this study included green tea (33%) and oolong tea (5%) which contained certain amounts of caffeine and polyphenol, our blend-tea had been expected to show a certain positive effect on obesity-related disorders. However, the present study found that the blend-tea did not have any detectable effects on adipose tissue weight, plasma insulin level or hepatic triglyceride level. The possibility that the doses of the blend-tea used in this study were too low to act on obesity cannot be excluded.

In the present study, TACC mitigated obesity in mice. Caffeine has been reported to promote lipolysis in the adipocytes of not only mice (43), but also humans (44). Arginine has also been shown to stimulate the secretion of glucagon in humans (10), leading to enhanced lipolysis in adipose tissue. Therefore, the anti-obesity effect of TACC should be expected to occur in humans. Our preliminary study suggests a decrease of percent body fat in healthy young women after daily intake of TACC-supplemented tea. More detailed studies on humans and animals are currently underway in our laboratory.

In conclusion, we have shown that intake of TACC-supplemented tea combined with dietary intervention effectively reduced adipose tissue mass in association with improved hyperinsulinemia and fatty liver in KK mice. Our results imply that TACC is a beneficial agent for the mitigation of obesity.

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