

Anti-obesity Effects of Three Major Components of Green Tea, Catechins, Caffeine and Theanine, in Mice

GUODONG ZHENG¹, KAZUTOSHI SAYAMA¹, TSUTOMU OHKUBO²,
LEKH RAI JUNEJA² and ITARO OGUNI³

¹Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka-shi 422-8529; ²Central Research Laboratories, Taiyo Kagaku Co., Ltd., 1-3 Takaramachi, Yokkaichi-shi 510-0844; ³School of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Shizuoka-shi 422-8526, Japan

Abstract. To elucidate the anti-obesity effects of three major components of green tea, catechins, caffeine and theanine, female ICR mice were fed on diets containing 2% green tea powder and diets containing 0.3% catechins, 0.05% caffeine and 0.03% theanine, which correspond, respectively, to their concentrations in a 2% green tea powder diet, singly and in combination for 16 weeks. Body weight and food intake were determined monthly during this period, kidneys, adrenals, liver, spleen, brain, pituitary and intraperitoneal adipose tissues (IPAT) were weighed and lipid levels in the serum and liver were measured at the end of this period. The body weight increase and weight of IPAT were significantly reduced by the diets containing green tea, caffeine, theanine, caffeine + catechins, caffeine + theanine and caffeine + catechins + theanine. Noticeably, the IPAT weight decreased by 76.8% in the caffeine + catechins compared to the control group. Serum concentrations of triglycerides (TG) and non-esterified fatty acids (NEFA) were decreased by green tea, catechins and theanine. Moreover, caffeine + catechins, caffeine + theanine and caffeine + catechins + theanine also decreased NEFA in the serum. The TG level in the liver was significantly reduced by catechins and catechins + theanine in comparison with the control. These results indicated that at least caffeine and theanine were responsible for the suppressive effect of green tea powder (GTP) on body weight increase and fatty acid accumulation. Moreover, it was shown that catechins and caffeine were synergistic in anti-obesity activities.

Corresponding to: Dr. Kazutoshi Sayama, Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka-shi 422-8529, Japan. Tel: +81-54-238-4865, e-mail: acksaya@agr.shizuoka.ac.jp

Key Words: Green tea powder, catechins, caffeine, theanine, fatty acid accumulation; lipid metabolism, anti-obesity.

Green tea is one of the most popular beverages consumed worldwide. It has been reported that green tea and its components have many biological and biochemical effects such as anti-mutation (1), anti-carcinogenesis (2-4), anti-oxidation (5), apoptosis-inducing (6) and anti-angiogenesis (7). Moreover, epidemiological studies have implied that green tea drinking reduces blood cholesterol in Japanese (8). Recently, we reported that the addition of 2% green tea powder (GTP) to the diet suppressed fat accumulation and body weight increase without reduction of food intake in mice (9). However, it is not clear what components of green tea are responsible for its anti-obesity activities.

Green tea has many components such as catechins, caffeine, theanine and vitamins (10). Catechins are present from 15% to 20% by weight in green tea. Green tea catechins have a hypocholesterolemic effect (11,12) and suppress the intestinal absorption of cholesterol (11,13). Moreover, it was reported that epigallocatechin gallate (EGCG), a kind of catechin, had an inhibitory effect on acetyl-CoA carboxylase which is essential for fatty acid biosynthesis *in vitro* (14) and anti-obesity effects at high doses in rats (15,16). Caffeine is the most effective anti-obesity component in oolong tea (17,18). It was shown that caffeine decreased food intake (19) and increased thermogenesis and that the thermogenic effect induced the body weight reduction (20). Moreover, it was clarified that the thermogenesis by caffeine was synergistically enhanced with catechins in rat adipose tissues (21). Theanine (γ -glutamylethylamide) is a main amino acid peculiar to green tea and has physiological effects such as relaxation activity (22), activation of dopamine metabolism and release in the brain (23). Moreover, it was reported that theanine suppressed excitation by caffeine (24). Therefore, we supposed that the physiological effects of catechins, caffeine and theanine might be concerned with the anti-obesity effect of green tea.

Thus, to clarify what components of green tea are responsible for the anti-obesity effects of a 2% green tea

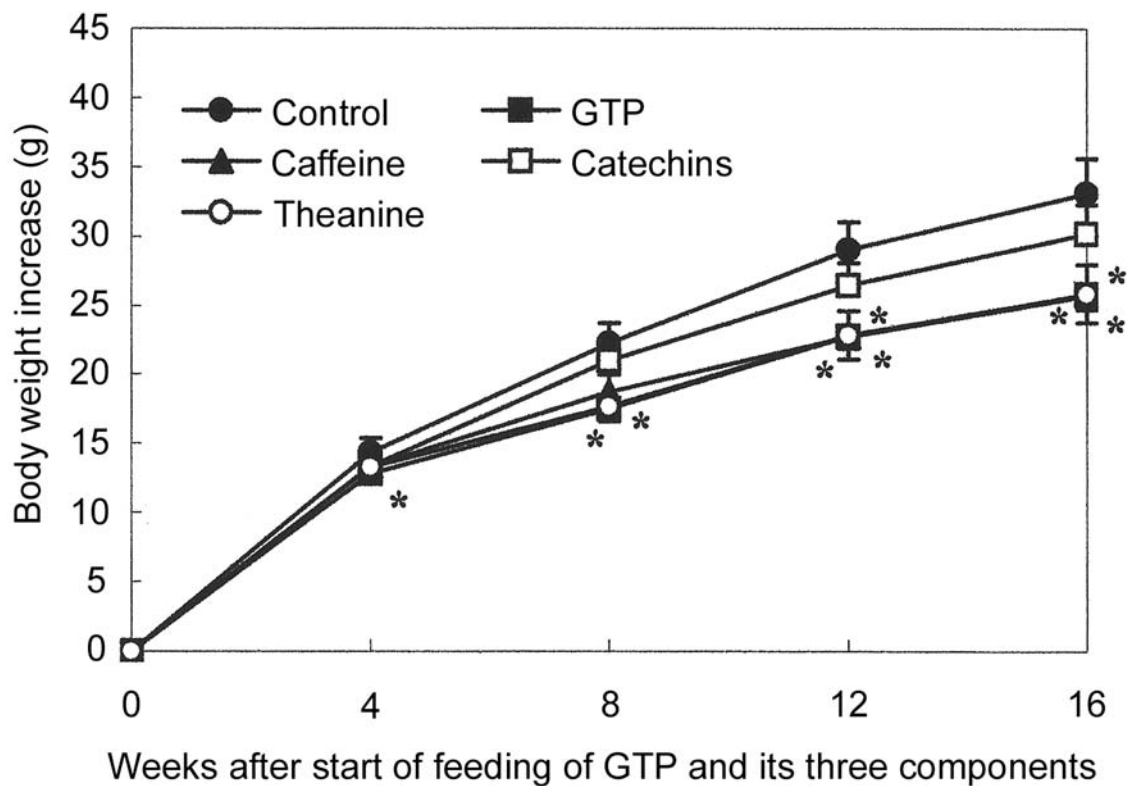


Figure 1. Effects of green tea powder (GTP) and its components on the body weight increase in mice. The means and SE for 10 mice are plotted. *: Significant difference at $p < 0.05$ compared to the control.

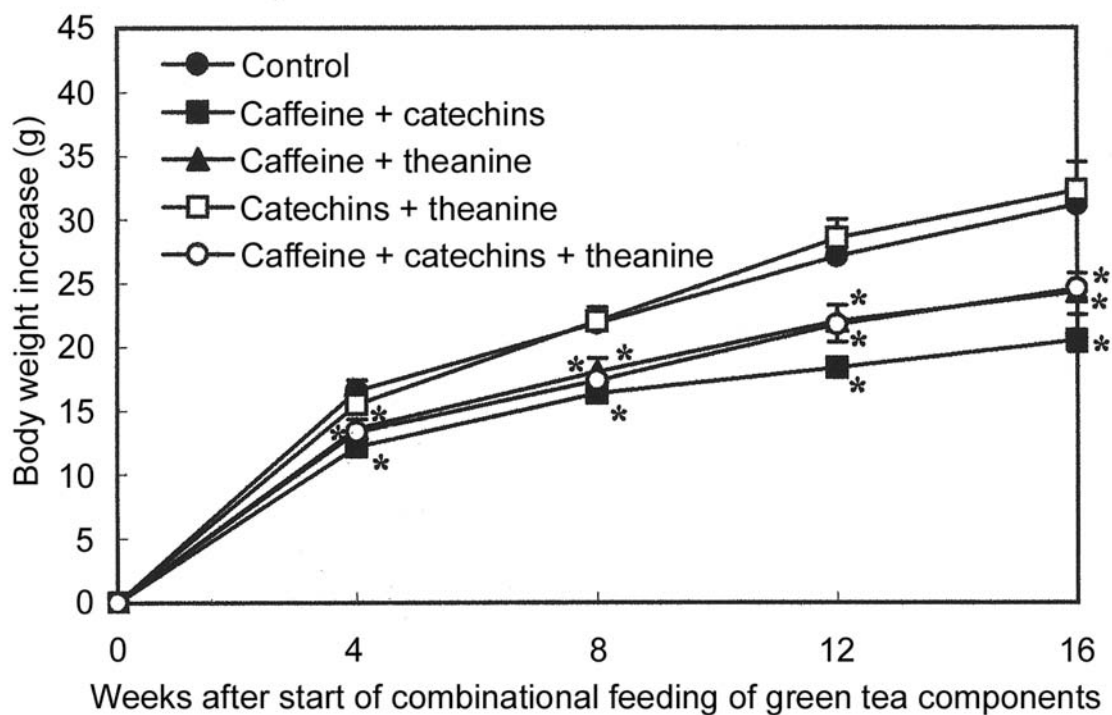


Figure 2. Effects of combinations of green tea components on the body weight increase in mice. The means and SE for 10 mice are plotted. *: Significant difference at $p < 0.05$ compared to the control.

Table I. Effects of green tea powder (GTP) and its components on weights of organs and intraperitoneal adipose tissues (IPAT) in mice.

	Control	GTP	Caffeine	Catechins	Theanine
Liver (g)	1.62±0.06 ^a	1.52±0.07	1.71±0.09	1.62±0.06	1.51±0.06
Kidneys (mg)	491.9±13.9	491.6±10.0	526.8±15.7	499.8±13.0	503.1±15.2
Spleen (mg)	161.8±8.4	159.0±8.6	162.0±8.8	132.0±6.1 ^b	142.0±10.4
Brain (mg)	538.5±8.1	516.8±5.3	531.1±6.3	524.0±6.0	520.9±14.9
Adrenals (mg)	11.89±1.57	12.57±0.49	14.81±0.73 ^b	12.75±0.75	11.56±0.79
Pituitary (mg)	3.04±0.13	3.10±0.10	2.98±0.22	2.83±0.24	2.71±0.17
IPAT(g)	6.59±0.83	3.16±0.39 ^b	3.47±0.74 ^b	6.01±0.67	3.84±0.53 ^b

^a: Values are means±SE for 10 mice.

^b: A significant difference at $p<0.05$ compared to the control.

Table II. Combinational effects of green tea components on weight of organs and intraperitoneal adipose tissues (IPAT) in mice.

	Control	Caffeine + catechins	Caffeine + theanine	Catechins + theanine	Caffeine + theanine + catechins
Liver (g)	1.71±0.05 ^a	1.59±0.08	1.75±0.05	1.68±0.05	1.72±0.10
Kidneys (mg)	549.1±16.5	479.2±10.9 ^b	550.4±12.1	540.0±23.4	535.4±18.8
Spleen (mg)	184.5±26.0	157.0±13.7	176.5±12.8	156.5±10.0	163.5±6.5
Brain (mg)	530.8±4.2	530.5±3.4	538.9±7.0	536.6±5.9	543.0±7.1
Adrenals (mg)	13.79±1.06	13.60±0.42	12.71±0.27	12.54±0.54	12.42±0.54
Pituitary (mg)	3.13±0.14	3.09±0.15	3.39±0.15	3.18±0.09	2.93±0.11
IPAT (g)	5.12±0.62	1.19±0.26 ^b	2.44±0.49 ^b	5.96±0.74	2.75±0.66 ^b

^a: Values are means±SE for 10 mice.

^b: A significant difference at $p<0.05$ compared to the control.

diet, we investigated the effects of three major components of green tea, caffeine, catechism and thiamine, alone or in combination on the weights of body and several organs, food intake and lipid levels in mice.

Materials and Methods

Animals and diets. Four-week-old female ICR mice weighing approximately 18g, were purchased from an animal breeder (Japan SLC Co. Hamamatsu, Japan) and used in this study. Green tea powder (*Camellia sinensis*, Yabukita Cha in Japanese) and crude catechins and theanine extracted from green tea were gifted by Kisaku-en Co. (Shizuoka, Japan) and Taiyo Kagaku Co. (Yokkaichi, Japan), respectively. Caffeine was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Green tea powder (GTP) was mixed with a commercial powder diet for mice (Oriental Yeast Co., Tokyo, Japan) at a concentration of 2%. Catechins, caffeine and theanine were mixed with the power diet at concentrations of 0.3%, 0.05% and 0.03%, respectively, singly and in combination. These concentrations correspond to those of catechins, caffeine and theanine in a 2% green tea powder diet, respectively. One hundred mice were divided at random into 10 groups of 10 mice each and fed on the mixed or non-mixed diets and tap water *ad libitum* for 16 weeks. During this feeding period, the body weight of each mouse was measured every month. At the end of this period, all mice were fasted for 12 h prior to sacrifice

for the determination of lipids in the serum and liver. After the fasting, each mouse was deeply anesthetized by an overdose of chloroform and the blood was drawn from the heart. Serum was isolated from the clotted blood by centrifugation at 825xg for 20 min at 4°C. Moreover, kidneys, adrenals, liver, spleen, brain, pituitary and intraperitoneal adipose tissue were weighed in individual mice. Sera and livers were all stored at -80°C until use for determination of the lipid levels.

The food intake was measured as follows: a group of 6 mice were individually housed in mouse cages and fed on a mixed or non-mixed diet. The food intake of each mouse was measured every other day and the amount of the food consumed was calculated every 4 weeks. All mice were kept in an air-conditioned (temperature: 24±1.0°C, and humidity: 40-60%) and light-controlled (14L:10D, light on from 06:00 to 20:00) animal room.

Lipid analyses. The serum concentrations of total cholesterol (TC), TG, phospholipid (PL), non-esterified fatty acids (NEFA) were measured enzymatically with test kits (cholesterol C-test, triglyceride G-test and phospholipid B-test and NEFA C-test, Wako Co., Japan). The total lipids were extracted from liver by the method of Folch *et al.* (25). TC, TG and PL in the extract were analyzed by the methods of Zak (26), Fletcher (27) and Bartlett (28), respectively.

Statistical analysis. Student's *t*-test was used for statistical evaluation and the differences were considered significant at $p<0.05$.

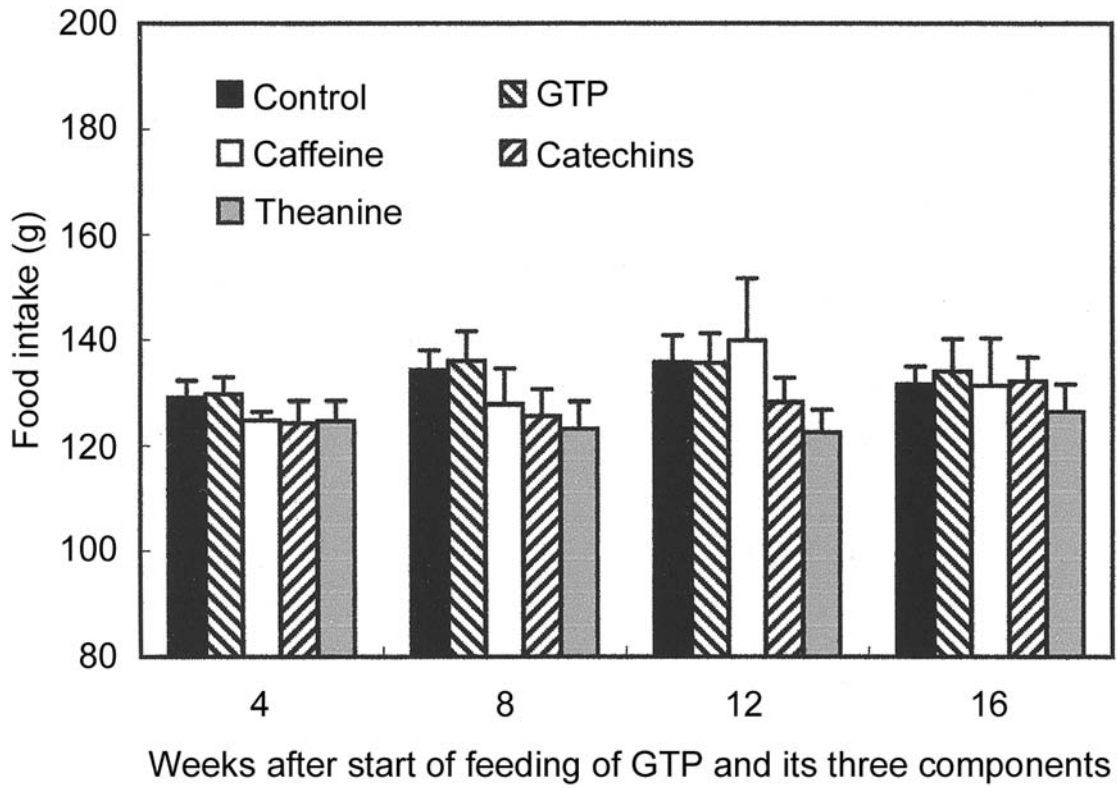


Figure 3. Effects of green tea powder (GTP) and its components on the food intake in mice. The means and SE for 6 mice are plotted.

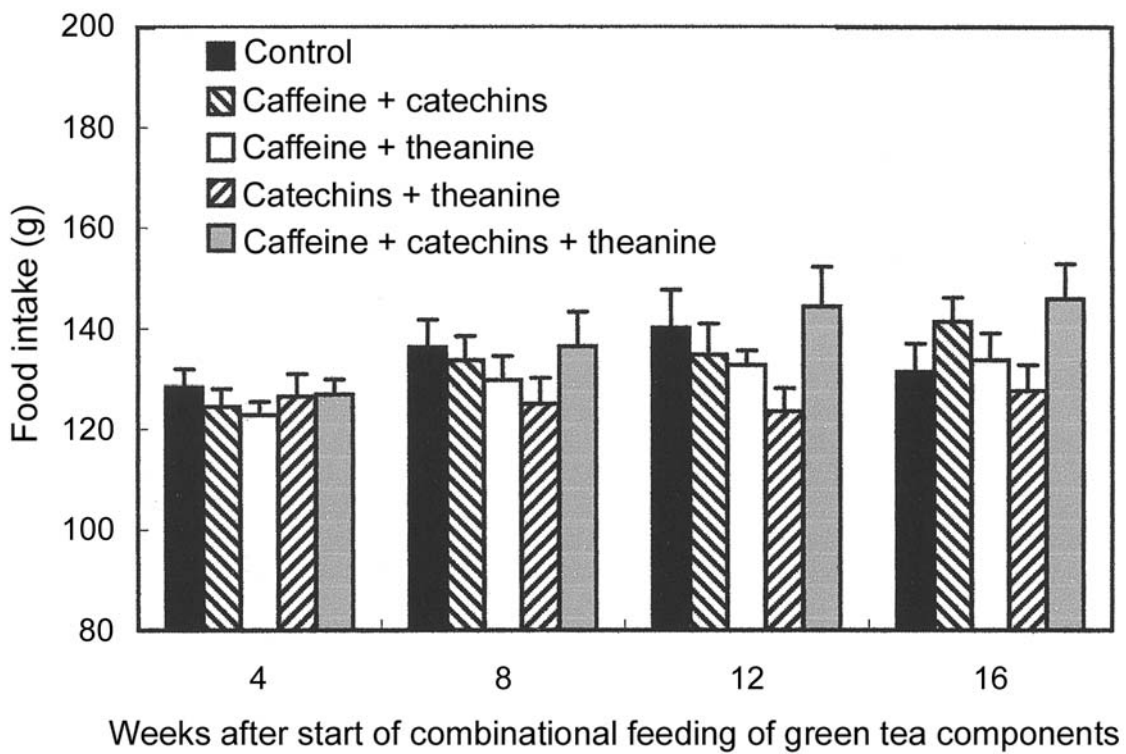


Figure 4. Effects of combinations green tea components on the food intake in mice. The means and SE for 6 mice are plotted.

Table III. Effects of green tea powder (GTP) and its components on the lipids levels in serum and liver in mice.

	Control	GTP	Caffeine	Catechins	Theanine
Serum lipids					
TC (g/l)	1.23±0.11 ^a	1.21±0.11	1.41±0.11	1.14±0.09	1.09±0.08
TG (g/l)	1.70±0.17	1.21±0.11 ^b	1.58±0.11	1.25±0.05 ^b	1.18±0.11 ^b
PL (g/l)	1.54±0.04	1.67±0.08	1.67±0.12	1.45±0.09	1.53±0.10
NEFA (mEq/l)	1.86±0.08	1.41±0.09 ^b	1.82±0.10	1.31±0.10 ^b	1.36±0.10 ^b
Liver lipids (μmol/g liver weight)					
TC	24.61±0.98	23.00±0.94	25.06±0.66	23.23±0.40	24.64±0.76
TG	30.66±0.96	28.59±1.48	30.25±1.25	26.76±1.42 ^b	29.00±1.51
PL	26.91±1.27	25.89±0.78	26.39±1.21	26.38±1.14	25.80±0.83

TG: Total cholesterol, TG: Triglycerides, PL: Phospholipids, NEFA: Non-esterified fatty acids.

^a: Values are means±SE for 10 mice.

^b: A significant difference at $p < 0.05$ compared to the control.

Table IV. Combinational effects of green tea components on the lipids level in serum and liver in mice.

	Control	Caffeine + catechins	Caffeine + theanine	Catechins + theanine	Caffeine + theanine + catechins
Serum lipids					
TC (g/l)	0.91±0.07 ^a	0.85±0.06	0.96±0.08	0.91±0.05	0.87±0.04
TG (g/l)	1.16±0.11	0.99±0.07	1.10±0.09	1.10±0.11	0.95±0.10
PL (g/l)	1.44±0.09	1.54±0.08	1.55±0.10	1.59±0.06	1.45±0.07
NEFA (mEq/l)	2.00±0.12	1.58±0.08 ^b	1.58±0.05 ^b	1.70±0.08	1.33±0.07 ^b
Liver lipids (μmol/g liver weight)					
TC	35.66±1.64	32.93±1.54	32.28±1.60	32.37±1.60	31.81±1.87
TG	16.40±1.02	14.47±0.62	14.86±0.64	13.22±0.65 ^b	14.00±0.75
PL	39.83±1.32	38.67±0.77	38.90±1.42	39.39±1.17	37.53±0.78

TG: Total cholesterol, TG: Triglycerides, PL: Phospholipids, NEFA: Non-esterified fatty acids.

^a: Values are means±SE for 10 mice.

^b: A significant difference at $p < 0.05$ compared to the control.

Results

Body and organ weights. The body weights of mice administered with GTP, catechins, caffeine and theanine are shown in Figure 1. The body weight gain was significantly suppressed by GTP, caffeine and theanine diets from the 4th, 12th and 8th week until the end of feeding, respectively, but not by the catechins diet. Figure 2 shows the synergistic effects of catechins, caffeine and theanine on the body weight in mice. The body weight increase was significantly suppressed by GTP, caffeine + catechins, caffeine + theanine and caffeine + catechins + theanine diets from the 4th week until the end of experiment. Particularly, the diets containing GTP and caffeine + catechins remarkably reduced the body weight. The weights of adrenals, kidneys, liver, spleen, brain, pituitary and intraperitoneal adipose tissues (IPAT) determined at the end of feeding of the diets containing

GTP, catechins, caffeine and theanine are presented in Table I. The weight of IPAT was remarkably reduced by the diets containing GTP, caffeine and theanine, by 48.0%, 52.7% and 58.3%, respectively, but not influenced by catechins. The weight of adrenals increased in the caffeine group and the spleen weight decreased in the catechins group significantly, but not so remarkably as compared with the control group. Table II shows the weights of organs and IPAT in the mice given catechins, caffeine and theanine in combination. The weight of IPAT was remarkably (23.2%, 47.6% and 53.7%) reduced by the diets containing caffeine + catechins, caffeine + theanine and caffeine + catechins + theanine, respectively. The kidney weight was significantly but not so remarkably decreased in the caffeine + catechins group as compared with the control group. The results showed that caffeine and theanine had anti-obesity activities. Moreover, it was indicated that the combination of caffeine and catechins was the most

effective among the green tea components in the suppression of body weight gain and fatty accumulation.

Food intake. Since GTP and its three components had a significant suppressive effect on the body weight (Figures 1 and 2), the food intake was compared between the group given them singly and the control group. As shown in Figure 3, the feeding of GTP, catechins, caffeine and theanine did not affect the food intake at all. The same results were obtained when catechins, caffeine and theanine were mixed with the diet in combination (Figure 4).

Concentrations of lipids in serum and liver. Table III summarizes the concentrations of lipids in the serum and liver after administration of GTP and its three components. The concentrations of triglycerides (TG) and non-esterified fatty acids (NEFA) in the sera from the mice fed GTP, catechins and theanine diets were significantly lower than those in the control. The level of TG in the liver was remarkably reduced by the catechins diet. The levels of lipids in the serum and liver after administration of catechins, caffeine and theanine in combination are presented in Table IV. The NEFA concentration in the serum was significantly lower in the groups fed caffeine + catechins, caffeine + theanine and caffeine + catechins + theanine diets than the control group. Moreover, the level of TG in the liver was remarkably reduced by the catechins + theanine diet.

Discussion

The present results clearly demonstrated that body weight increase and fat accumulation in mice were remarkably reduced by the diets added with caffeine, theanine, caffeine + catechins, caffeine + theanine and caffeine + catechins + theanine. Particularly, the body weight increase was most remarkably reduced in the caffeine + catechins group among all groups and the IPAT weight was decreased by 23.2% in this group compared with the control. These results indicated that at least caffeine and theanine were responsible for the suppressive effect of GTP on the body weight increase and fatty accumulation. Moreover, it was shown that catechins acted synergistically with caffeine in manifestation of anti-obesity activities.

It has been reported that caffeine ingestion elevated the metabolic rate and fat oxidation *in vivo* through lipolysis in fat cells and the release of catecholamines (29-31). Moreover, caffeine enhanced noradrenaline- or adrenaline-induced lipolysis in fat cells (17,32). We also obtained results which supported the anti-obesity activities of caffeine. Thus, it seems that the anti-obesity effect of caffeine in GTP was due to enhancement of thermogenesis and fat metabolism.

The diet containing 0.3% catechins did not influence fat accumulation and body weight increase in mice. However, it was reported that catechins significantly inhibited TG accumulation and synthesis in 3T3-L1 cells (14). Murase *et al.* (33) clarified that a diet containing tea catechins at 0.2% showed an anti-obesity action in mice at 27 weeks of feeding although the body weight was significantly lower in mice fed a 0.5% tea catechins diet at 12 weeks of feeding. Moreover, EGCG, a kind of catechins, significantly reduced or prevented body weight gain with reduction of food intake in lean and obese rats when it was intraperitoneally injected at a daily dose of 81 to 92mg/kg for 7 days (15). These reports indicated that catechins have an anti-obesity potential. We also showed that the levels of TG and NEFA in the serum and TG level in the liver were decreased by catechins and previously reported that GTP had an anorectic effect and was more effective in reduction of body weight increase and fat accumulation when added to the diet at 4% rather than at 2% concentration (9). Thus, catechins might exhibit anti-obesity action at a higher dose.

Although catechins did not show anti-obesity effects, the combination of caffeine and catechins induced stronger suppression of the body weight increase and fat accumulation than caffeine alone. The suppressive effect of caffeine and catechins in combination was strongest in all experimental groups and almost equal to the effect achieved by GTP addition. Dulloo *et al.* (34) reported that thermogenesis and fat oxidation were synergistically enhanced by catechins and that both green tea components might be applied to the management of obesity. Our results supported the hypothesis strongly and indicated that it might be possible to prevent obesity by continuous and long-term administration of caffeine and catechins.

On the other hand, we demonstrated that theanine also had anti-obesity action. It was reported that theanine could pass through the blood-brain barrier and induced increase of dopamine release (22) and reduction of serotonin concentration in the brain (35, 36). It has been reported that several neurotransmitters such as dopamine and serotonin are involved in the regulation of food intake (37). Amphetamine, a drug that increases brain dopamine concentration, has anorexigenic effects (38). In this study, the food intake tended to decrease in the mice given theanine. Moreover, dopaminergic agonists such as SKF-38393 and bromocriptine improved body weight gain in genetically obese ob/ob mice by normalizing the elevated levels of both neuropeptide Y and corticotrophin-releasing hormone (39). Therefore, the anti-obesity effect of theanine might be caused by changes of neurotransmitters in the brain. However, theanine did not augment the anti-obesity action of caffeine and/or catechins.

Addition of GTP to the diet decreased serum TG and NEFA levels in mice. TG and NEFA levels in the serum were also significantly reduced by administration of

catechins or theanine. It was reported that EGCG decreased the serum TG level in rats (15). Thus, the reduction of serum lipid levels by green tea might be caused by the catechins and theanine contained in green tea.

More extensive studies are in progress to clarify the mechanism how GTP and its components suppress lipid metabolism and fat accumulation.

Acknowledgements

This research was partly supported by Goto Research Grant from the University of Shizuoka, Japan. The authors are grateful to Professor Akio Matsuzawa, Institute of Medical Science, Tokyo University, Japan, for his kind advice.

References

- Okuda T, Mori K and Hayatsu H: Inhibitory effect of tannins on direct-acting mutation. *Chem Pharm Bull* 32: 3755-3758, 1984.
- Oguni I, Nasu K, Kanaya S, Ota Y, Yamamoto S and Nomura T: Epidemiological and experimental studies on the antitumor activity by green tea extract. *Jpn J Nutr* 47: 93-102, 1989.
- Hara Y, Matsuzaki S and Nakamura K: Anti-tumor activity of green tea catechins. *J Jpn Soc Nutr Food Sci* 42: 39-45, 1989.
- Valcic S, Timmermann BN, Aiberts DS, Wachter GA, Krutzsch M, Wymer J and Guillen JM: Inhibitory effect of six green tea catechins and caffeine on the growth of four selected human tumor cell lines. *Anti-cancer Drugs* 7: 461-468, 1996.
- Matsuzaki T and Hara Y: Antioxidative activity of tea leaf catechins. *Nippon Nogekigaku Kaishi* 59: 129-134, 1985.
- Ahmad N, Fayes DK, Nieminen AL, Agarwal R and Mukhtar H: Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *J Nat Cancer Inst* 89: 1881-1889, 1997.
- Cao Y and Cao R: Angiogenesis inhibited by drinking tea. *Nature* 398: 1999, 1999.
- Kono S, Shinchi K, Wakabayashi K, Honjo S, Todoroki I, Sakurai Y, Imanishi K, Nishizawa H, Ogawa S and Katsurada M: Relation of green tea consumption to serum lipids and lipoproteins in Japanese men. *J Epidemiol* 6: 128-133, 1996.
- Sayama K, Lin S, Zheng G and Oguni I: Effects of green tea on growth, food utilization and lipid metabolism in mice. *In Vivo* 14: 481-484, 2000.
- Takeo T: Green and semi-fermented teas. *In: Willson KC and Clifford MN (ed), Tea: Cultivation to Consumption*, Chapman and Hall, London and Tokyo, pp.413-457, 1992.
- Muramatsu K, Fukuyo M and Hara Y: Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J Nutr Sci Vitaminol* 32: 613-622, 1986.
- Fukuyo M, Hara Y and Muramatsu K: Effect of tea leaf catechin, (-)-epigallocatechin gallate, on plasma cholesterol level in rats. *J Jpn Soc Nutr Food Sci* 39: 495-500, 1986.
- Ikeda I, Imasato Y, Sasaki E, Nakayama M, Nagano H, Takeo T, Yayabe F and Sugano M: Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *Biochem Biophys Acta* 1127: 141-146, 1992.
- Watanabe J, Kawabata J and Niki R: Isolation and identification of acetyl-CoA carboxylase inhibitors from green tea (*Camellia sinensis*). *Biosci Biotechnol Biochem* 62: 532-534, 1998.
- Kao YH, Hiipakka RA and Liao S: Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. *Endocrinology* 141: 980-987, 2000.
- Kao YH, Hiipakka RA and Liao S: Modulation of obesity by a green tea catechin. *Am J Clin Nutr* 72: 1232-1234, 2000.
- Han LK, Takaku T, Li J, Kimura Y and Okuda H: Anti-obesity action of oolong tea. *Int J Obes* 23: 98-105, 1999.
- Rumpler W, Seale J, Clevidence B, Judd J, Wiley E, Yamamoto S, Komatsu T, Sawaki T, Ishikura Y and Hosoda K: Oolong tea increases metabolic rate and fat oxidation in men. *J Nutr* 131: 2848-2852, 2001.
- Racotta IS, Leblanc J and Richard D: The effect of caffeine on food intake in rats: Involvement of corticotropin-releasing factor and the sympatho-adrenal system. *Pharm Biochem Behav* 48: 887-892, 1994.
- Dulloo AG, Geissler CA, Horton T, Clooins A and Miller DS: Normal caffeine consumption: influence on thermogenesis and daily energy expenditure in lean and postobese human volunteers. *Am J Clin Nutr* 49: 44-50, 1989.
- Dulloo AG, Seydoux J, Girardier L, Chantre P and Vandermander J: Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *Int J Obes* 24: 252-258, 2000.
- Kobayashi K, Nagato Y, Aoi N, Juneja LR, Kim M, Yamamoto T and Sugimoto S: Effects of L-theanine on the release of α -brain waves in human volunteers. *Nippon Nogekigaku Kaishi* 72: 153-157, 1998.
- Yokogoshi H, Kobayashi M, Mochizuki M and Terashima T: Effect of theanine, γ -glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats. *Neurochem Res* 23: 667-673, 1998.
- Kakuda T, Nozawa A, Unno T, Okamura N and Okai O: Inhibiting effects of theanine on caffeine stimulation evaluated by EEG in the rat. *Biosci Biotechnol Biochem* 64: 287-293, 2000.
- Folch J, Lees M and Sloane-Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 479-509, 1957.
- Zak B: Simple rapid microtechnic for serum total cholesterol. *Am J Clin Pathol* 27: 583-588, 1957.
- Fletcher MJ: A colorimetric method for estimating serum triglycerides. *Clin Chim Acta* 22: 393-397, 1968.
- Bartlett GR: Phosphorus assay in column chromatography. *J Biol Chem* 234: 466-468, 1959.
- Jung RT, Shetty PS, Jame WPT, Barrand MA and Callingham BA: Caffeine: its effect on catecholamine and metabolism in lean and obese humans. *Clin Sci* 60: 527-535, 1981.
- Arciero PJ, Gradner AW, Calles-Escandon J, Benowitz NL and Poehlman ET: Effects of caffeine ingestion on NE kinetics, fat oxidation, and energy expenditure in younger and older men. *Am J Physiol* 268: E1192-1198, 1995.
- Robertson D, Frolich JC, Carr RK, Watson JT, Hollifield JW, Shand DG and Oates JA: Effects of caffeine on plasma rennin activity, catecholamines and blood pressure. *N Engl J Med* 298: 181-186, 1978.
- Dulloo AG, Seydoux J and Girardier L: Potentiation of the thermogenic antiobesity effects of ephedrine by dietary methylxanthines, adenosine antagonism or phosphodiesterase inhibition? *Metabolism* 41: 1233-1241, 1992.
- Murase T, Nagasawa A, Suzuki J, Hase T and Tokimitsu I: Beneficial effects of tea catechins on diet-induced obesity:

- stimulation of lipid catabolism in the liver. *Int J Obesity* 26: 1459-1464, 2002.
- 34 Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, Chantre P and Vandermander J: Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutr* 70: 1040-1045, 1999.
- 35 Yokogoshi H, Mochizuki M and Saitoh K: Theanine-induced reduction of brain serotonin concentration in rats. *Biosci Biotech Biochem* 62: 816-817, 1998.
- 36 Yokogoshi H, Kato Y, Sagesaka Y, Matsuura T, Kakuda T and Takeuchi N: Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. *Biosci Biotech Biochem* 59: 615-618, 1995.
- 37 Schwartz MW, Woods SC, Porte D Jr, Seeley RJ and Baskin DG: Central nervous system control of food intake. *Nature* 404: 661-671, 2000.
- 38 Towell A, Muscat R and Willer P: Behavioural microanalysis of the role dopamine in amphetamine anorexia. *Pharmacol Biochem Behav* 30: 641-648, 1988.
- 39 Bina KG and Cincotta AH: Dopaminergic agonists normalize elevated hypothalamic neuropeptide Y and corticotropin-releasing hormone, body weight gain, and hyperglycemia in ob/ob mice. *Neuroendocrinology* 71: 68-78, 2000.

Received September 15, 2003
Accepted December 12, 2003

Editorial Office: International Institute of Anticancer Research,
1st km Kapandritiou - Kalamou Rd., Kapandriti, P.O.B. 22, Attiki 19014, Greece
Fax:0030-22950-53389;Tel.: 0030-22950-52945; e-mail: journals@iiar-anticancer.org

Please type or print the requested information on the reprint order form and return it to the Editorial Office by fax or e-mail.

Reprints must be paid for in advance.

If your paper is subject to charges for excess pages or color plates, please add these charges to the payment for reprints.

The reprints are not to be sold.

PRICE LIST FOR REPRINTS WITHOUT COVER

Page length	Number of copies requested									
	100	200	300	400	500	1000	1500	2000	3000	5000
1-4pp EURO	335	387	438	503	554	851	1135	1470	2038	3225
5-8	438	503	580	645	722	1083	1445	1832	2554	4012
9-12	554	619	709	787	877	1341	1780	2219	3096	4824
13-16	709	787	890	993	1096	1625	2141	2657	3676	5715
17-20	838	929	1032	1148	1277	1883	2451	3044	4244	6527

For reprints with cover: Please add EURO 140.00 per 100 copies.

Postage: Please add 4% on the above prices.

Reprint Order Form

Of my paper No. **426-Z** comprising **8** printed pages, entitled «**Anti-obesity Effects of Three Major...**» accepted for publication in **in vivo** Vol. **18** No. **1**

- I require a total of _____ copies at EURO _____
- I do not require reprints
- Please send me a copy of this issue containing my paper at EURO 45.00
- Please enter my personal subscription to **in vivo** at the special Author's price of EURO 390.00
(Year: 2004)
- A check for the above amounts payable to J. G. Delinassios, Executive Publisher of Anticancer Research Journal, is enclosed.
- Please send an invoice to:

For EC countries: Please give your VAT number:

City and Date:

Exact postal address:

Tel:

Fax:

Signature: