Effects of the Long-Term Ingestion of Tea Catechins on Energy Expenditure and Dietary Fat Oxidation in Healthy Subjects

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The long-term ingestion of tea catechins has been reported to reduce body fat. The aim of this study was to investigate the effect of the long-term ingestion of tea catechins on postprandial energy expenditure and dietary fat oxidation. Twelve healthy men aged 27–48 years participated in the study. The subjects consumed 350 ml of a test beverage/day that contained either a high dose of catechin (592.9 mg) or a low dose of catechin (77.7 mg) for a period of 12 weeks. Respiratory analyses were conducted before and at 4, 8, and 12 weeks during the test period, in which oxygen consumption and the excretion of $^{13}CO_2$ were monitored over 8 hr after a single ingestion of a test meal containing ^{13}C labeled triglyceride. The excretion of $^{13}CO_2$ in the high dose catechin group (the HC group) was significantly increased at 4 and 12 weeks of the test period compared to that for the low dose catechin group (the LC group) ($p < 0.05$), and this elevation persisted at 8.9% at week 0 to 12.9% at week 12. Dietary induced thermogenesis (DIT), defined as an increased energy expenditure from the fasting baseline for 8 hr after the single ingestion of a test meal, was significantly higher in the HC group at 8 and 12 weeks compared to that in the LC group ($p < 0.05$) with elevation to 90.3 kcal at week 12 from 51.4 kcal at week 0. In conclusion, enhanced dietary fat oxidation and an increased DIT may play an important role in the mechanism of the anti-obesity effect of tea catechins.

Key words —— tea catechin, diet induced thermogenesis, fat oxidation, energy expenditure

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INTRODUCTION

Green tea contains about 10–20 percent of low molecular weight polyphenols, which are mainly comprised of flavonoid monomers (flavan-3-ol) referred to as tea catechins. The physiological activities of tea catechins have been investigated and reported to include the antioxidative and anticancer activities.[1,2)]

Tsuchida *et al*. recently reported on the effects of tea catechin on body fat.[3)] In a randomized, parallel 12-week clinical study, healthy men [mean body mass index (BMI) of 26.5] and postmenopausal women (mean BMI of 25.9) ingested a beverage containing either 588 mg of tea catechins in the treatment group or 126 mg of tea catechins in the control group. A significant reduction in abdominal fat was observed in the catechin treatment group, despite the fact that the total energy intakes was equal in both groups throughout the test period, suggesting the possible enhanced energy expenditure and/or inhibition of the absorption of ingested material, following the long-term ingestion of tea catechins.

Dulloo *et al*. demonstrated that the single ingestion of tea catechins led to an increased energy expenditure in humans.[4)] Murase *et al*. reported that the long-term feeding of tea catechins increased acyl-CoA oxidase and medium chain acyl-CoA dehydrogenase mRNA expression as well as beta-oxidation activity in the livers of mice,[5)] suggesting that the long-term consumption of tea catechins may affect energy expenditure and fat oxidation.

In addition, Laville *et al*. investigated diet-induced thermogenesis (DIT) in obese or non-obese subjects, as assessed by the underwater weight method.[6)] They demonstrated a significant lower DIT in obese subjects compared to non-obese subjects, suggesting that a defective DIT response could lead to weight gain over a period of time. Marques-Lopes *et al*. also reported that obese subjects had a signifi-

cantly lower fat oxidation derived from DIT, whereas a higher accumulation of body fat derived from dietary fat.[7)]

Based on these findings, we investigated the impact of the long-term intake of tea catechins on resting and postprandial energy expenditure. The metabolic rate of dietary fat was also investigated by feeding ^{13}C-labeled triacylglycerol.

MATERIALS AND METHODS

Test Beverage and Test Meal Containing a Stable Isotope —— The tea catechins used as the test beverage in the present study were prepared by the method reported by Yayabe *et al*.[8)] Test beverages were prepared in the form of a 350 ml sized drink containing two different dosages of tea catechins: a low dose (including 77.7 mg catechins) and a high dose (including 592.9 mg catechins). The tea catechins in the test beverages were analyzed by the method of Saijo *et al*.[9)] Table 1 shows the composition of the tea catechins.

Table 1. Tea Catechins and Other Composition of Test Beverages

	High dose	Low dose
catechin	11.7	1.7
epicatechin	8.6	1.3
gallocatechin	37.9	6.0
epigallocatechin	21.0	2.3
catechin gallate	10.8	1.1
epicatechin gallate	9.4	1.1
gallocatechin gallate	39.9	5.1
epigallocatechin gallate	3.1	3.4
total catechin	169.4	22.2
caffeine	23.4	22.9

(mg/100 ml)

^{13}C labeled triacylglycerol, used as a probe for dietary fat, was synthesized from [1–^{13}C] labeled palmitic acid and free glycerol by the method described by Watanabe *et al*.[10)] Each subject consumed 900 mg of ^{13}C-labeled triacylglycerol as part of the test meal in the single meal ingestion study.

Subjects and Experimental Design —— Twelve healthy men, aged 27 to 48 years, whose body mass index (BMI) was classified in the range of normal or obese class 1 by the definition of the Japan Society for the Study of Obesity, were enrolled. Prior to ingestion of the test beverage, anthropometric measurements, a single meal ingestion study and a dietary intake analysis, assessed by a consecutive 7-day diet record, were performed as a screening process. The subjects were divided into two groups; a low dose catechin group (the LC group, $n = 6$) and a high dose catechin group (the HC group, $n = 6$) so that the anthropometrics, dietary intake and respiratory parameters listed in Table 2 were matched between the two groups. The baseline characteristics of the subjects are shown in Table 2, indicating the absence of any statistical differences between the groups.

The subjects were requested to ingest test beverages every morning for a period of 12 weeks. Single test meal ingestion studies to obtain respiratory data were conducted at 4, 8 and 12 weeks of the test period. During the test period, subjects were instructed to maintain their usual dietary and exercise habits, except for the ingestion of the test beverage. The subjects were also requested to submit a consecutive 7-day diet record one week prior to each single test meal ingestion study to monitor their energy intake. This study was conducted in accordance with the Helsinki Declaration and informed consent was obtained from all subjects.

Respiratory Analysis —— The single test meal ingestion studies for the collection of respiratory data

Table 2. Characteristics of Subjects before Test Beverage Administration

	HC ($n = 6$)	LC ($n = 6$)
age	37.7 ± 2.6	38.0 ± 2.9
BMI (kg/m^2)	24.8 ± 0.8	24.6 ± 0.8
waist (cm)	85.0 ± 1.6	85.8 ± 2.9
calorie intake (kcal/day)	2064 ± 144	2041 ± 81
fat intake (g/day)	69.3 ± 6.2	69.2 ± 7.5
oxygen consumption (l/8 hr)	2.18 ± 0.13	2.18 ± 0.11
respiratory quotient	5.47 ± 0.14	5.67 ± 0.34
Atom %	8.74 ± 0.01	8.73 ± 0.01

(mean ± SEM)

were conducted a total of four times, before and after 4, 8 and 12 weeks of the test period. One day prior to the single test meal ingestion study, subjects ate a special meal (800 kcal, carbohydrate 105 g, protein 40 g, fat 25 g) at designated time between 18:00 and 19:00 and were allowed to drink only plain water thereafter. On the day of the single ingestion study, the subjects did not ingest any test beverage and fasting was maintained. They came to the laboratory at 8:00 and a baseline respiratory analysis was performed, while maintaining a sedentary posture for 30 min. ^{13}C labeled triacylglycerol, used as a probe for dietary fat, was spread on bread as part of an 800 kcal test meal (carbohydrate 110 g, protein 26 g, fat 30 g) and subjects ate the material at 9:00. A respiratory analysis was conducted to measure oxygen consumption and the excretion of CO_2 at 1, 2, 3, 4, 6 and 8 hr after ingestion of the test meal. In addition to the respiratory analysis, breath samples were separately collected in aluminous bags to monitor the level of $^{13}CO_2$ excretion. Each measurement was maintained for 20 min. After 10 min of resting in a sedentary condition, the subjects were fitted with facemasks for about 2 min to ensure stable breathing. Data were then recorded for the next seven minutes. The collected data were averaged and expressed as integration values of postprandial variations. Oxygen consumption was measured using VO2000 (S&ME, Inc.; Nakano-ku, Tokyo, Japan). The level of ^{13}C label excreted as $^{13}CO_2$ was measured by means of a continuous flow isotope ratio mass spectrometry (ANCA, NT System Corp.;Osaka city, Osaka, Japan). The level of $^{13}CO_2$ excreted was calculated according to the method described by Murphy *et al*. as follows[11]:

$$\text{Atom\%} = {}^{13}CO_2/({}^{12}CO_2 + {}^{13}CO_2) \times 100$$

$$^{13}C \text{ excretion to } ^{13}C \text{ administrated ratio} = (\text{Atom\%}^T - \text{Atom\%}^B) \times VCO_2{}^T \div ({}^{13}CW \times 22.4)$$

where $\text{Atom\%}^T$ denotes the Atom% at each time point, $\text{Atom\%}^B$ denotes Atom% at baseline, $VCO_2{}^T$ denotes the excretion of carbon dioxide at each time point, ^{13}CW denotes the molecular weight of administrated ^{13}C.

The total ^{13}C label excreted as $^{13}CO_2$ for 8 hr after the test meal ingestion was extrapolated as the integration value of "the ^{13}C excretion to ^{13}C administrated ratio" from hours 0 to 8.

Evaluation of Abdominal Fat Area —— At the beginning of the test period, subjects underwent computed tomography scanning at the umbilicus level (Foundation for the detection of early gastric carcinoma, Chuo-ku, Tokyo, Japan) using a tube voltage of 120 kVp and a tube current of 250 mAs. The images were analyzed using a window width of 400 and window level of –10, 0, or +10. Visceral fat areas were calculated from the CT images obtained at the umbilicus level employing the visceral fat measurement PC software, Fat Scan Ver. 2 (N2 system Corp.; Osaka city, Osaka, Japan) and evaluated as the abdominal fat area.

Statistical Analysis —— Data were expressed as the mean ± SEM. Statistically significant differences in the time-course measurements for each group were examined by the paired *t*-test, in which significant differences in intergroup measurements were examined by the *t*-test. The correlations between variables were tested by Pearson's correlation coefficient. A level of confidence of $p < 0.05$ was considered to be significant.

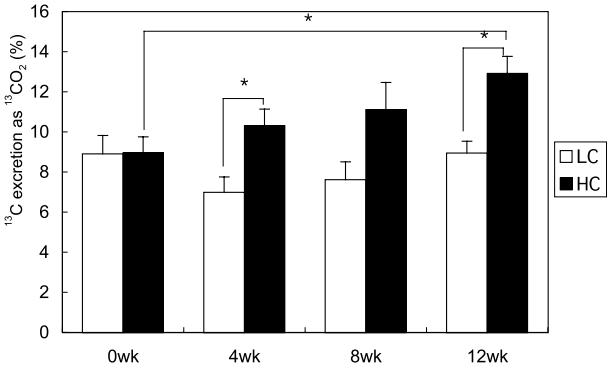

Fig. 1. Excretion of ^{13}C in the Breath as $^{13}CO_2$ Over 8 hr at 0, 4, 8 and 12 weeks of the Test Period

Results are expressed as the mean ± SEM. *$p < 0.05$. LC = the low dose catechin group (open columns, $n = 6$). HC = the high dose catechin group (closed columns, $n = 6$).

RESULTS AND DISCUSSION

The purpose of the study was to investigate the impact of the long-term ingestion of tea catechins on postprandial energy expenditure and dietary fat oxidation. Dietary fat oxidation was measured by the ^{13}C-label excreted as $^{13}CO_2$ in the breath over an 8 hr period after the ingestion of a single meal. Figure 1 shows data relation to $^{13}CO_2$ excretion over 8 hr at various time points of the test periods. The HC group showed a significantly higher $^{13}CO_2$ excretion compared to the LC group at weeks 4 and 12 ($p < 0.05$), accompanied by a continuous increase throughout the test period with a significant difference between week 0 (8.9%) and week 12 (12.9%) ($p < 0.05$). To the contrary, no such enhancement was

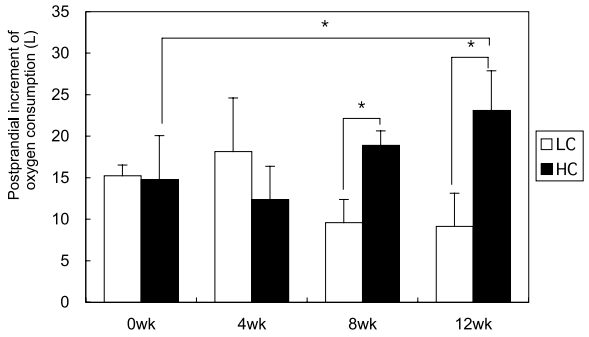

Fig. 2. The Postprandial Increment of Oxygen Consumption by the Fasting State Over 8 hr at 0, 4, 8 and 12 weeks of the Test Period

Results are expressed as the mean ± SEM. *$p < 0.05$. LC = the low dose catechin group (open columns, $n = 6$). HC = the high dose catechin group (closed columns, $n = 6$).

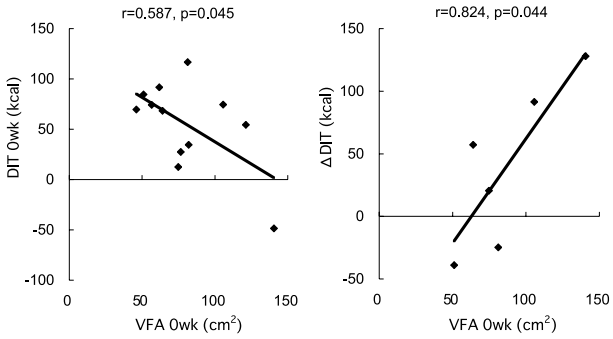

Fig. 3. A, Relationship between the Veseral Fat Area (VFA) at week 0 and DIT at week 0 for All Subjects ($r = 0.587$, $p = 0.045$)

B, Relationship between veseral fat area at week 0 and the change in DIT for the experimental period for the HC group (ΔDIT) ($r = 0.824$, $p = 0.044$).

observed in the LC group. The extent of $^{13}CO_2$ excretion at week 0 in the present study was similar to results that were previously reported by Murphy *et al*.[11] These results suggest that the continuous ingestion of tea catechin for 12 weeks enhances the oxidation of dietary fat as measured by ^{13}C-label excreted as $^{13}CO_2$.

With respect to postprandial energy expenditure, Fig. 2 shows the effect of the long-term ingestion of tea catechins on the increment of oxygen consumption after ingestion of a single test meal. Oxygen consumption over 8 hr after the single ingestion of the test meal was significantly higher in the HC group than in the LC group at weeks 8 and 12 of the test period ($p < 0.05$). The difference in oxygen consumption between the HC and LC groups was more evident at week 12. In the present study, DIT is defined as the postprandial increment of energy expenditure from fasting over 8 hr after the single ingestion of the test meal.[12] In the HC group, the DIT was increased from 51.4 kcal in week 0 to 90.3 kcal in week 12. However, the oxygen consumption at baseline for the HC group remained unchange, which is considered to be the basal metabolic rate even after the 12-week ingestion (data not shown).

Daily energy expenditure consists of three components: basal metabolic rate, 60–75%; energy expenditure due to physical activity, about 20%; DIT, about 10%.[13] Although DIT contributes to a small portion of total energy expenditure, Laville *et al*. reported that a significantly lower DIT in obese subjects might have induced obesity.[6] In addition, Thomas *et al*. reported a significantly decreased in postprandial fat oxidation in obese subjects.[14] In the present study, we found a negative correlation between the initial abdominal visceral fat area and DIT prior to the administration of the test beverages ($r = 0.59$, $p < 0.05$) as shown in Fig. 3A, indicating a lower DIT in subjects with a larger abdominal visceral fat area.

On the other hand, Fig. 3B indicates a significant positive correlation between the initial abdominal fat area and changes in DIT during a test period of 12 weeks ($r = 0.82$, $p < 0.05$) in the HC group. It can thus be speculated that long-term ingestion of tea catechins stimulates DIT more efficiently in obese subjects, where fat metabolism should be lowered initially. These findings are in general agreement with results reported by Hase *et al*.,[15] in which anti-obesity effects of the long-term ingestion of tea catechin was more evident in subjects with a higher BMI in terms of metabolic energy aspects.

The increment of DIT from the baseline was 21.2 kcal/8 hr at week 8 and 38.9 kcal/8 hr at week 12. If it is assumed that the DIT increases at every meal, the total increment of energy expenditure was estimated to be 5000 to 9000 kcal in 12 weeks, presumably resulting in an approximate weight reduction of 1 kg. Several previous studies, in which a 12-week ingestion of tea catechins at a dose of 480–580 mg/day resulted in 1.0–1.3 kg of weight loss,[3,15] supports this assumptions. Based of these findings, enhanced DIT after the long-term ingestion of tea catechin may play an important role in the body fat reduction observed in earlier studies.

Murase *et al*.[5] investigated the effect of the long-term administration of tea catechins on lipid metabolism using diet-induced obese mice, in which the

long-term feeding of tea catechins increased beta-oxidation activity in the liver, in combination with enhanced expressions of m-RNA relating to beta-oxidation, including acyl-CoA oxidase and medium chain acyl-CoA dehydrogenase. These results are consistent with an increase in dietary fat oxidation and DIT as the result of the long-term ingestion of tea catechins. Further investigations will be needed to clarify the detailed mechanism of this effect.

In summary, the findings herein demonstrate an enhancement in dietary fat oxidation and DIT as the result of the long-term ingestion of tea catechin. These results provide a new insight for clarifying the mechanism by which tea catechins to prevent obesity by altering energy metabolism.

REFERENCES

1) Yoshino, K., Hara, Y., Sano, M. and Tomita, I. (1994) Antioxidative effects of black tea theaflavins and thearubigin on lipid peroxidation of rat liver homogenates induced by tert-butyl hydroperoxide. *Biol. Pharm. Bull*., **17**, 146–149.
2) Agarwal, R., Katiyar, S. K., Zaidi, S. I. and Mukhtar, H. (1992) Inhibition of skin tumor promoter-caused induction of epidermal ornithine decarboxylase in SENCAR mice by polyphenolic fraction isolated from green tea and its individual epicatechin derivatives. *Cancer Res*., **52**, 3582–3588.
3) Tsuchida, T., Itakura, H. and Nakamura, H. (2002) Reduction of body fat in humans by long-term ingestion of catechins. *Prog. Med*., **22**, 2189–2203.
4) Dullo, A. G., Duret, C., Rohrer, D., Girardier, L., Mensi, N., Fathi, M., Chantre, P. and Vandermander, J. (1999) Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-hr energy expenditure and fat oxidation in humans. *Am. J. Clin. Nutr*., **70**, 1040–1045.
5) Murase, T., Nagasawa, A., Suzuki, J., Hase, T. and Tokimitsu, I. (2002) Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. *Int. J. Obes. Relat. Metab. Disord*., **26**, 1459–1464.
6) Laville, M., Cornu, C., Normand, S., Mithieux, G., Beylot, M. and Riou, J. P. (1993) Decreased glucose-induced thermogenesis at the onset of obesity. *Am. J. Clin. Nutr*., **57**, 851–856.
7) Marques-Lopes, I., Ansorena, D., Astiasaran, I., Forga, L. and Martinez, J. A. (2001) Postprandial de novo lipogenesis and metabolic changes induced by a high-carbohydrate, low-fat meal in lean and overweight men. *Am. J. Clin. Nutr*., **73**, 253–261.
8) Yayabe, F., Kinugasa, H. and Takeo, T. (1989) A simple preparative chromatographic separation of green tea catechins. *Nippon Nogeikagaku Kaishi*, **63**, 845–847.
9) Saijo, R. and Takeda, Y. (1999) HPLC analysis of catechins in various kinds of green teas produced in Japan and abroad. *Nippon Nogeikagaku Kaishi*, **46**, 138–147.
10) Watanabe, T., Shimizu, M., Sugiura, M., Sato, M., Kohori, J., Yamada, N. and Nakanishi, K. (2003) Diacylglycerol using immobilized 1,3-regiospecific lipase lipozyme RM IM. *J. Am. Oil Chem. Soc*., **80**, 1201–1207.
11) Murphy, J. L., Jones, A., Brookes, S. and Wootton, S. A. (1995) The gastrointestinal handling and metabolism of [1-13C]palmitic acid in healthy women. *Lipids*, **30**, 291–298.
12) D'Alessio, D. A., Kavle, E. C., Mozzoli, M. A., Smalley, K. J., Polansky, M., Kendrick, Z. V., Owen, L. R., Bushman, M. C., Boden, G. and Owen, O. E. (1983) Thermic effect of food in lean and obese men. *J. Clin. Invest*., **81**, 1781–1789.
13) Granata, G. P. and Brandon, L. J. (2002) The thermic effect of food and obesity: discrepant results and methodological variations. *Nutr. Rev*., **60**, 223–233.
14) Thomas, C. D., Peters, J. C., Reed, G. W., Abumrad, N. N., Sun, M. and Hill, J. O. (1992) Nutrient balance and energy expenditure during ad libitum feeding of high-fat and high-carbohydrate diets in humans. *Am. J. Clin. Nutr*., **55**, 934–942.
15) Hase, T., Komine, Y., Meguro, S., Takeda, Y., Takahashi, H., Matsui, Y., Inaoka, S., Katsuragi, Y., Tokimitsu, I., Shimasaki, H. and Itakura, H. (2001) Anti-obesity effects of tea catechins in humans. *J. Oleo. Sci*., **50**, 63–69.