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*Am J Physiol Regulatory Integrative Comp Physiol* 288:708-715, 2005. First published Nov 24, 2004; doi:10.1152/ajpregu.00693.2004

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T. Murase, S. Haramizu, A. Shimotoyodome, I. Tokimitsu and T. Hase

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**Green tea extract and its major polyphenol (-)-epigallocatechin gallate improve muscle function in a mouse model for Duchenne muscular dystrophy**

O. M. Dorchies, S. Wagner, O. Vuadens, K. Waldhauser, T. M. Buetler, P. Kucera and U. T. Ruegg

*Am J Physiol Cell Physiol*, February 1, 2006; 290 (2): C616-C625.

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# Green tea extract improves endurance capacity and increases muscle lipid oxidation in mice

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**Murase, Takatoshi, Satoshi Haramizu, Akira Shimotoyodome, Azumi Nagasawa, and Ichiro Tokimitsu.** Green tea extract improves endurance capacity and increases muscle lipid oxidation in mice. *Am J Physiol Regul Integr Comp Physiol* 288: R708–R715, 2005. First published November 24, 2004; doi:10.1152/ajpregu.00693.2004.—Green tea contains a high level of polyphenolic compounds known as catechins. We investigated the effects of green tea extract (GTE), which is rich in catechins, on endurance capacity, energy metabolism, and fat oxidation in BALB/c mice over a 10-wk period. Swimming times to exhaustion for mice fed 0.2–0.5% (wt/wt) GTE were prolonged by 8–24%. The effects were dose dependent and accompanied by lower respiratory quotients and higher rates of fat oxidation as determined by indirect calorimetry. In addition, feeding with GTE increased the level of  $\beta$ -oxidation activity in skeletal muscle. Plasma lactate concentrations in mice fed GTE were significantly decreased after exercise, concomitant with increases in free fatty acid concentrations in plasma, suggesting an increased lipid use as an energy source in GTE-fed mice. Epigallocatechin gallate (EGCG), a major component of tea catechins, also enhanced endurance capacity, suggesting that the endurance-improving effects of GTE were mediated, at least in part, by EGCG. The  $\beta$ -oxidation activity and the level of fatty acid translocase/CD36 mRNA in the muscle was higher in GTE-fed mice compared with control mice. These results indicate that GTE are beneficial for improving endurance capacity and support the hypothesis that the stimulation of fatty acid use is a promising strategy for improving endurance capacity.

catechins; epigallocatechin gallate; lipid metabolism; swimming

UNDERSTANDING EXERCISE PHYSIOLOGY is important both for the maintenance of our health and for sports training programs. Many obesity- and lifestyle-related diseases are caused by a lack of exercise and by disturbance of eating habits (21). Therefore, regular exercise and improvement in our diet are highly recommended for disease prevention (27, 36). In addition, lipid and carbohydrate metabolism have major effects both on the development of obesity and exercise endurance (13, 44, 46), suggesting that energy metabolism is a key factor in both cases.

Skeletal muscle uses carbohydrates, lipids, and amino acids as energy sources, but the ratio in which they are used varies with the intensity of exercise and the level of fitness (12, 17). During aerobic exercise, both free fatty acids and glucose are important energy sources. However, during endurance exercise, excess glucose is undesirable, because it induces insulin secretion, which in turn simultaneously inhibits lipid metabolism and stimulates lactate production. Conversely, enhanced availability and use of free fatty acids are considered to reduce carbohydrate use, which in turn spares glycogen and sup-

presses lactate production and results in an increase in endurance. On the basis of this theory, many attempts have been made to improve endurance capacity by nutritional regimens that promote fatty acid oxidation (11, 18). So far, the effects of a high-fat diet, medium-chain triacylglycerol, caffeine, L-carnitine, and capsaicin have been investigated; however, the efficacy of dietary interventions is still controversial.

Green tea and cacao contain a class of polyphenols known as catechins, which consists mainly of epigallocatechin gallate (EGCG), epicatechin gallate, and gallic acid gallate. Catechins have been reported to possess various biological and pharmacological properties, including antiatherogenic (3, 28), antibacterial (40), and anticarcinogenic activities (48). We (30) have recently demonstrated that the long-term consumption of tea catechins was beneficial in counteracting the obesity-inducing effects of a high-fat diet and that their effects may be attributed, at least in part, to the activation of hepatic lipid catabolism in C57BL/6 mice. Dulloo et al. (8) reported that the ingestion of green tea extract (GTE) with caffeine increased energy expenditure and decreased the respiratory quotient (RQ) in humans. Overall, observations so far suggest that thermogenesis and fat oxidation are stimulated by the intake of tea catechins.

To confirm our hypothesis that catechins affect endurance exercise capacity (i.e., time to exhaustion) by increasing lipid use, in this study, we examined the effect of catechin-rich GTE intake on the endurance capacity of BALB/c mice swimming in an adjustable-current water pool. We also analyzed changes in energy metabolism, especially lipid metabolism. We demonstrated that GTE intake improved endurance capacity, and this was accompanied by an increase in lipid catabolism. Our results support the hypothesis that stimulation of lipid metabolism is a promising strategy for improving the capacity for endurance training.

## MATERIALS AND METHODS

### GTE

GTE was prepared and analyzed as described previously (31). In brief, green tea leaves (*Camellia sinensis*) were extracted with hot water and the extract was reduced to a powder using the spray-dry method. The extract was then dissolved in hot water, and mixed with an equal volume of chloroform. The aqueous phase was recovered with three volumes of ethanol, and the extract was freeze-dried after removing the solvent. The composition of catechins was measured by HPLC. Total catechin content in the GTE was 81% (the sum of each of the catechins described below). The caffeine content was 0.1%. The composition of isolated tea catechins was EGCG (41%), epigallocate-

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echin (23%), epicatechin gallate (12%), epicatechin (9%), gallic acid (7%), gallic acid gallate (4%), and others (4%). EGCG (98%) (Teavigo) was purchased from DMS Nutritional Products (Tokyo, Japan). No caffeine was detectable in Teavigo.

#### Animals and Diets

**Experiment 1.** Four-week-old male BALB/c mice obtained from Charles River (Kanagawa, Japan) were maintained at  $23 \pm 2^\circ\text{C}$  under a 12:12-h light-dark cycle (lights on from 0700 to 1900). At 8 wk of age, initial measurements of endurance capacity for swimming were made, and the mice were divided into four groups ( $n = 10$ , 5 mice/cage). All mice were allowed unlimited access to water and a synthetic diet containing (in %) 10 (wt/wt) fat, 20 casein, 55.5 potato starch, 8.1 cellulose, 2.2 vitamins, 0.2 methionine, and 4 minerals; control animals were fed this diet alone and experimental animals had this diet supplemented with 0.2 and 0.5% GTE. The animals were maintained on their respective diets for 10 wk. During this period, experimental mice were exercised in a pool twice a week as described below, but the nonexercise (non-Ex)-control mice were not. During the experiments, the animals were cared for in accordance with the American Physiological Society Guiding Principles in the Care and Use of Animals. This study was approved by the Animal Care Committee of Kao Tochigi Institute.

**Experiment 2.** Eight-week-old mice were divided into four groups ( $n = 10$ , 5 mice/cage) and were allowed unlimited access to the synthetic diet used in *experiment 1*, but in this case, the experimental groups received a diet containing 0.1–0.5% EGCG for 10 wk. During this period, experimental mice were exercised in a pool twice a week, as described below. On the final day of the experiment, all mice were killed, and each gastrocnemius muscle was rapidly dissected for use in Northern blot analysis.

#### Swimming Exercise and Evaluation of Endurance Capacity

An adjustable-current water pool was used to determine the endurance capacity for swimming as previously reported (25, 29). Briefly, we used an acrylic pool (90 cm long  $\times$  45 cm wide  $\times$  45 cm deep) filled with water to a depth of 38 cm. The current in the pool was generated with a pump (type C-P60H; Hitachi, Tokyo, Japan), and the strength of the current was adjusted by opening and closing a valve. The current speed at the surface was measured with a digital current meter (model SV-101–25S; Sankou, Tokyo, Japan) at the start of every swimming session, and we confirmed the current was at a constant speed. The water temperature was maintained at  $34^\circ\text{C}$  with an electric heater.

In preliminary training sessions, mice were accustomed to swimming for 30 min, three times a week, at a 6 l/min flow rate. After training, mice were fasted for 2 h before swimming, and then their maximum swim times were measured at a flow rate of 7 l/min three times a week. To reduce the inherent variation in swimming capacity, we eliminated those mice whose mean maximum swim times were 40% longer or shorter than the average swim time of all mice. We also eliminated mice whose maximum swim times varied greatly within three measurements. With the use of these criteria, 40 of 98 mice were selected and divided into experimental groups with similar swim times and body weights before the experiments.

We measured the endurance capacity of the mice, swimming in a flow rate of 7 l/min, once a week for the 10-wk experimental period. A mouse was deemed to be fatigued when it failed to rise to the surface of the water to breathe and was rescued at this point.

#### Body Weight and Food Intake

Body weight was measured weekly throughout the study. Food intake was recorded for each cage throughout the study.

#### Blood Analysis

On the final day of the 10-wk experiments, blood was collected from nonfasted, anesthetized mice from the postcaval vein. Four days before the end of *experiment 1*, blood samples were collected from the tail vein from mice immediately after 30 min of exercise. Plasma triglycerides, nonesterified fatty acids (NEFA), and glucose concentrations were determined by using triglyceride E-test, NEFA-C test, and glucose CII test assay kits (WAKO, Osaka, Japan), respectively. Plasma lactate was measured by using Lactate Pro (Arkley, Kyoto, Japan).

#### Muscle, Liver, and Fat Weights

Tissues were dissected from each mouse and the weights of white adipose tissue (epididymal, perirenal, and retroperitoneal), muscle (gastrocnemius, plantaris, and soleus), and liver were measured.

#### $\beta$ -Oxidation Activity

$\beta$ -Oxidation activity was measured as described previously (39), with minor modification. Frozen mouse gastrocnemius muscles were thawed and homogenized on ice using a polytron homogenizer (Microtech, Chiba, Japan) in 250 mM sucrose, 1 mM EDTA in 10 mM HEPES (pH 7.2). Subcellular debris was removed by centrifugation at 7000 g for 5 min. Aliquots of the resultant supernatant, containing 50  $\mu\text{g}$  protein were used for assays in a final volume of 200  $\mu\text{l}$  containing (in mM) 50 Tris·HCl (pH 8.0), 40 NaCl, 2 KCl, 2  $\text{MgCl}_2$ , 1 DTT, 5 ATP, 0.2 L-carnitine, 0.2 NAD, 0.06 FAD, 0.12 CoA, and 3  $\alpha$ -cyclodextrin, and 0.1  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]-labeled palmitic acid, at  $37^\circ\text{C}$ . The reaction was started by adding the substrate and was terminated after 20 min, by adding 200  $\mu\text{l}$  0.6 N perchloric acid. After centrifugation, supernatants were extracted three times with 1 ml of *n*-hexane to remove residual [ $^{14}\text{C}$ ]-labeled palmitic acid. The radioactivity remaining in the aqueous phases was measured in a liquid scintillation counter (model 2550TR/LL; Packard).

#### RNA Extraction and Northern Blot Analysis

Total RNA was isolated from the mouse gastrocnemius muscles using Isogen (WAKO), according to the manufacturer's instructions. Aliquots of purified RNA (10  $\mu\text{g}$ ) were electrophoresed on 1% agarose/formaldehyde gels and blotted onto Hybond- $\text{N}^+$  membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Blots were prehybridized for 15 min at  $68^\circ\text{C}$  in QuikHyb hybridization solution (Stratagene, CA), and then hybridized with a  $^{32}\text{P}$ -labeled cDNA probes at  $68^\circ\text{C}$  for 1 h. Membranes were washed in  $2 \times \text{SSC}/0.1\%$  SDS at room temperature and again in  $0.1 \times \text{SSC}/0.1\%$  SDS at  $68^\circ\text{C}$  and then autoradiographed and analyzed with a BAS2500 bioimage analyzer (Fuji, Tokyo, Japan). The following cDNA probes were prepared by RT-PCR: fatty acid translocase (FAT)/CD36 (NM007643, nucleotides 817–1117), medium-chain acyl-CoA dehydrogenase (MCAD) (J02791, nucleotides 671–1199), peroxisome proliferator-activated receptor (PPAR)- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) (NM008904, nucleotides 1724–2024), and 36B4 (Z29530, nucleotides 338–594). cDNA probes were labeled with [ $\alpha$ - $^{32}\text{P}$ ]dCTP using Ready-To-Go DNA labeling beads (Amersham Pharmacia Biotech). The mRNA levels were expressed relative to the mRNA levels of the 36B4 gene. Normalized values were expressed as a percentage using the value of the Ex-control group as 100%.

#### Indirect Calorimetry

Energy metabolism studies were carried out during the last week of the experiments using individual open-circuit indirect calorimeters (Oxymax; Columbus Instruments, Columbus, OH). Mice were deprived of food for 12 h, allowed access to diet for 1 h, and then oxygen consumption and respiratory quotient were measured for 5 h. Data represent mean values over the last 3 h.

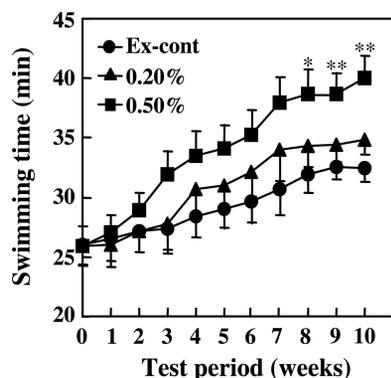


Fig. 1. Effects of green tea extract (GTE) on endurance capacity for swimming. Swimming time to fatigue was measured at a flow rate of 7 l/min. Each point represents the mean swimming time of 10 mice. Bars represent the SE. \* $P < 0.05$ , \*\* $P < 0.01$ , vs. the Ex-control group (mice exercised but not receiving GTE).

Lipid oxidation and carbohydrate oxidation were calculated by using the following equations (15)

$$\text{Lipid oxidation (mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\text{): } 1.67 \times (1 - \text{RQ}) \times \dot{V}\text{O}_2$$

$$\text{Carbohydrate oxidation (mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\text{): } (4.51 \times \text{RQ} - 3.18) \times \dot{V}\text{O}_2$$

Locomotor activity was measured by using the automated motion analysis system (SCANET model MV-10; Tokyo Sangyo, Toyama, Japan). This system consists of a cage equipped with two overlapping sensor frames at different heights, which detect small (M1) and large (M2) horizontal movements and vertical, rearing movements (RG) (26).

#### Statistical Analysis

All values are presented as means  $\pm$  SE. Comparisons of data were made by one-way ANOVA. When data were significant, each group was compared with the others by Fisher's protected least significant difference test (StatView; SAS Institute, Cary, NC). Results with values of  $P < 0.05$  were considered statistically significant.

## RESULTS

### Effects of GTE on Endurance Capacity for Swimming

It has previously been reported (29) that BALB/c mice are the most suitable strain for minimizing the variation in time taken to swim to the point of fatigue. Additionally, we have confirmed that these mice are resistant to diet-induced obesity (unpublished data). Therefore, to minimize the influence of changes in body fat mass on endurance capacity, we used this strain in the present study. Figure 1 shows the swimming times to exhaustion throughout the experimental period. After 10 wk, the swimming times for the exercise-only (Ex-control) mice

Table 2. Plasma analysis

	Non-Ex	Ex	Ex + 0.2% GTE	Ex + 0.5% GTE
Triglyceride, mg/dl	123.8 $\pm$ 11.9*	87.1 $\pm$ 7.1	98.6 $\pm$ 12.2	86.0 $\pm$ 8.4
NEFA, meq/l	1.04 $\pm$ 0.05*	0.85 $\pm$ 0.06	0.92 $\pm$ 0.09	0.84 $\pm$ 0.05
Glucose, mg/dl	201.6 $\pm$ 14.0	189.7 $\pm$ 7.4	184.0 $\pm$ 13.8	183.0 $\pm$ 9.7

On the final day of *experiment 1*, blood was collected under nonfasting, resting conditions. Values are means  $\pm$  SE. \* $P < 0.05$  vs. the Ex-control group. NEFA, nonesterified fatty acid.

were 25% longer than the initial times. Swimming times for mice fed 0.2 and 0.5% GTE at 10 wk of the experiment were 8 and 24% longer, respectively, compared with Ex-control mice, indicating that habitual GTE intake improved the endurance capacity for swimming.

### Effects of GTE on Tissue Weights

There was no significant difference in the average energy intake among the groups (Table 1). Compared with the non-Ex group, the weight of fat in mice that had habitual exercise was significantly lower. Supplementation with GTE tended to decrease adipose tissue weight dose dependently, and the retroperitoneal fat weight in mice fed 0.5% GTE was significantly lowered after 10 wk. No significant difference was observed in liver and muscle weight among experimental groups.

### Effects of GTE on Blood Components

There was no marked difference in plasma parameters between the Ex-control and GTE groups under resting conditions (Table 2). However, significant differences were observed in plasma NEFA and lactate immediately after exercise (Fig. 2). The plasma NEFA and lactate concentrations in the Ex-control group were higher than those in non-Ex group. Supplementation with GTE slightly, but significantly, increased the plasma NEFA concentrations. In contrast to NEFA, the increase in plasma lactate concentration in the exercised group was dose dependently suppressed by GTE. The plasma glucose levels in exercised groups were lower than that in the non-Ex group; however, there was no significant difference between the Ex-control and GTE groups (data not shown).

### Effects of GTE on Whole Body Energy Metabolism

We examined the effect of feeding GTE on energy metabolism in mice by monitoring oxygen consumption and the RQ. We began monitoring 2 h after a 1-h feeding period when the RQ

Table 1. Body weight, energy intake, muscle weight, and fat weight

	Non-Ex	Ex	Ex + 0.2% GTE	Ex + 0.5% GTE
Food intake, g	228.14 $\pm$ 3.02	220.14 $\pm$ 6.31	217.23 $\pm$ 4.42	216.69 $\pm$ 1.16
Final body weight, g	32.99 $\pm$ 0.39	31.59 $\pm$ 0.80	30.89 $\pm$ 0.48	30.64 $\pm$ 0.57
Liver, g	1.55 $\pm$ 0.04	1.50 $\pm$ 0.04	1.47 $\pm$ 0.04	1.46 $\pm$ 0.03
Gastrocnemius, mg	298 $\pm$ 6	288 $\pm$ 8	282 $\pm$ 7	288 $\pm$ 9
Plantaris, mg	32 $\pm$ 1	33 $\pm$ 1	32 $\pm$ 1	33 $\pm$ 2
Soleus, mg	18 $\pm$ 1	18 $\pm$ 1	17 $\pm$ 1	18 $\pm$ 1
Epididymal fat, mg	630 $\pm$ 49**	468 $\pm$ 35	420 $\pm$ 32	389 $\pm$ 15
Perirenal fat, mg	161 $\pm$ 12**	120 $\pm$ 9	106 $\pm$ 10	95 $\pm$ 10
Retroperitoneal fat, mg	182 $\pm$ 11*	144 $\pm$ 11	119 $\pm$ 15	107 $\pm$ 11*

Values are means  $\pm$  SE. \* $P < 0.05$ , \*\* $P < 0.01$ , vs. the Ex-control group. Ex, exercised; GTE, green tea extract.

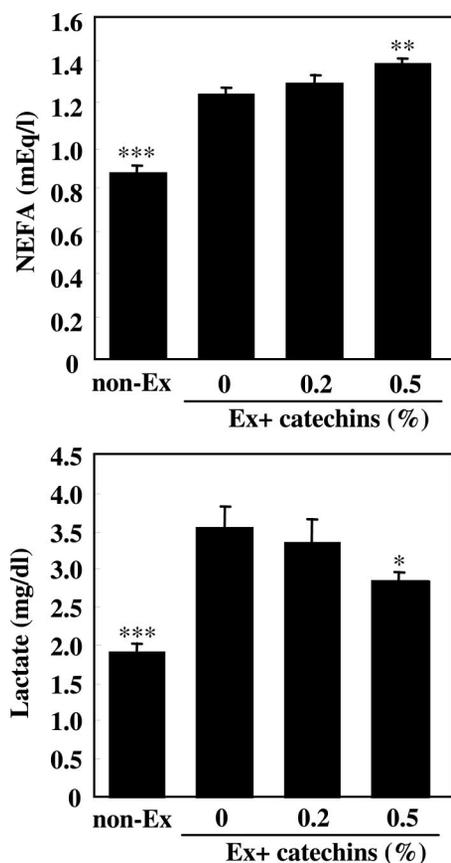


Fig. 2. Plasma nonesterified fatty acids (NEFA; A) and lactate (B) concentrations immediately after exercise. Plasma NEFA and lactate concentrations were measured immediately after 30 min of swimming at a flow rate of 7 l/min. Blood samples of the non-Ex (not exercised) group were collected under resting condition without exercise. Values are means  $\pm$  SE of 10 mice. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001, vs. the Ex-control group.

values were nearly 0.85. In the exercised groups, oxygen consumption was accelerated, RQ values were lowered, and the combustion of lipids was accelerated (Fig. 3). Oxygen consumption tended to increase in the groups fed GTE, but the differences were not significant. However, RQ values were lower, and fat oxidation was increased in the GTE-fed groups in a dose-dependent manner, suggesting an increased use of lipids to generate energy in these mice. There were no significant differences in locomotor activity among the experimental groups.

#### Effects of GTE on Fatty Acid $\beta$ -Oxidation

To elucidate the mechanisms underlying the effects of GTE, we examined fatty acid  $\beta$ -oxidation activity in gastrocnemius muscle. As shown in Fig. 4, habitual exercise significantly increased  $\beta$ -oxidation activity in muscle. A diet containing 0.5% GTE increased  $\beta$ -oxidation activity by 74 and 36% compared with the non-Ex-control and Ex-control groups, respectively, indicating that consumption of GTE stimulated lipid catabolism in the muscle.

#### Effects of EGCG on Endurance Capacity, $\beta$ -Oxidation Activity, and mRNA Expression of Genes Involved in Lipid Metabolism

We examined the effects of EGCG, a major component of GTE, and found that it dose dependently prolonged the swim-

ming time to exhaustion (Fig. 5). These results indicate that the endurance-enhancing activity of GTE is mediated, at least in part, by EGCG.

$\beta$ -oxidation activity in muscle was significantly increased by feeding with EGCG (Fig. 6), as well as with GTE (Fig. 4). We further examined the effects of EGCG on the mRNA expression of genes involved in lipid metabolism in muscle. Among the molecules examined in this study, marked changes were observed in FAT/CD36. In mice that were fed diets containing 0.2 and 0.5% EGCG, the FAT/CD36 mRNA levels in the muscle were significantly higher than that in mice that were fed the control diet (Fig. 7). When normalized to 36B4 mRNA levels, the FAT/CD36 mRNA levels in the 0.2 and 0.5% EGCG group were 43 and 54% higher, respectively, than in the control group. MCAD is a key molecule for mitochondrial  $\beta$ -oxidation, and its mRNA levels were highest in the 0.2% EGCG group. No marked changes were observed in the mRNA levels of PGC-1 $\alpha$ , an exercise-sensitive transcription regulator (42), under these experimental conditions.

#### DISCUSSION

In this study, we examined the effects of GTE on swimming time to exhaustion in mice and demonstrated that GTE markedly improved endurance capacity and stimulated lipid use. Because catechins are major components of GTE, this suggests that they make a crucial contribution to this effect.

Endurance capacity was improved by GTE intake without having a significant effect on body weight (Table 1), suggesting that prolonged time to fatigue is mainly due to the effect of GTE, and is not a secondary effect caused by the difference in buoyancy of body fat. During endurance exercise, skeletal muscle mainly relies on fat and carbohydrate for its energy (12, 17). It has been recognized that enhancing fatty acid oxidation during exercise reduces the rate of glycogen depletion, resulting in an improved endurance capacity. Therefore, in the search for strategies to improve athletic performance, recent investigations have focused on nutritional approaches that may theoretically promote fatty acid oxidation and so improve endurance capacity (11, 18). We have shown that GTE improved endurance capacity and that the improvement was dose dependent. A similar effect was observed in mice fed EGCG, a major constituent of GTE, suggesting that the effects of GTE were mediated, at least in part, by EGCG. However, because the effects of EGCG appear weak compared with those of GTE, we cannot rule out a possible contribution from other components of GTE. Although long-term intake of GTE enhanced endurance capacity, no marked effects were observed after a single dose of GTE (data not shown), suggesting that some biochemical changes induced by habitual GTE intake, such as upregulation of muscular  $\beta$ -oxidation, contribute to the improvement in endurance capacity.

Our results suggested that GTE improved endurance capacity by stimulating lipid catabolism and inducing a more effective use of lipids. Fat oxidation in indirect calorimetry was increased by GTE intake, indicating the increase of fat use at the individual level. Plasma NEFA measured immediately after exercise was slightly, but significantly, increased in mice fed tea catechins. The effect of plasma fatty acid level on endurance capacity is still controversial. However, an increased supply of fatty acids in the circulation would induce the uptake

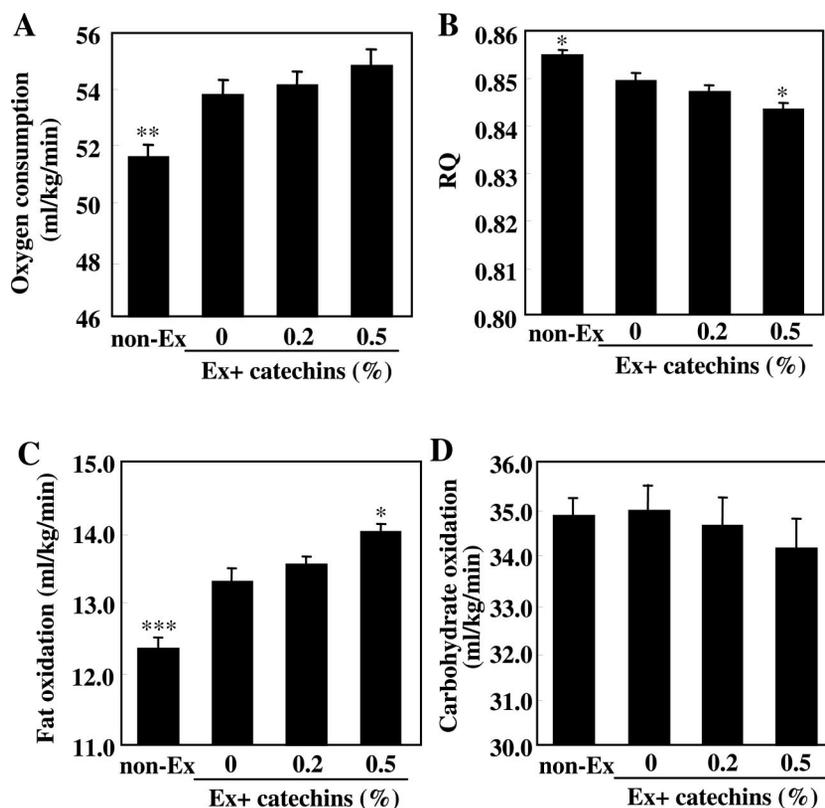


Fig. 3. Effects of GTE on whole body energy metabolism. Oxygen consumption (A) and respiratory quotients (RQ; B) were determined by indirect calorimetry. Fat oxidation (C) and carbohydrate oxidation (D) were calculated as described in MATERIALS AND METHODS. Values are means  $\pm$  SE of 10 mice. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. the Ex-control group.

of fatty acids, and thereby stimulate lipid metabolism in muscle (11, 18). As shown in Fig. 4, muscular  $\beta$ -oxidation activity was higher in mice fed GTE than in non-Ex and Ex-control mice, suggesting that GTE enhanced the capacity of muscle to catabolize lipids and use fatty acids as an energy source. Conversely, plasma lactate concentrations, which increase as a result of increased glycogen breakdown and glycolytic flux, were decreased by GTE. In separate experiments, we have observed that the decrease in muscular glycogen content seen after exercise was smaller in mice fed GTE than in Ex-control mice (unpublished observation). These results suggest that habitual exercise and the intake of GTE enhance fatty acid availability, catabolism, and use in muscle, and this is accompanied by a reduction in carbohydrate use, which together

result in prolonged swimming times to exhaustion. Wang et al. (46) reported recently that overexpression of PPAR $\delta$  in skeletal muscle led to an increase of endurance capacity together with upregulation of lipid metabolism-related molecules and changes of muscle fiber type. Moreover, in a preliminary experiment, we found that the PPAR $\delta$  agonist L-165401 significantly enhanced the endurance capacity of mice by 12% within as little as 10 days (data not shown). These results also support the view that muscular fatty acid metabolism is important in exercise performance.

Results of studies investigating the ergogenic effects of caffeine and the possible role of fatty acids are contradictory. Some studies have shown that caffeine affects muscle, adipose, and central nervous tissue indirectly by enhancing the release

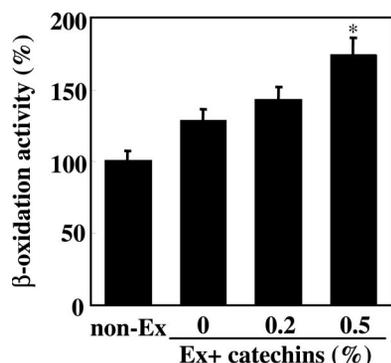


Fig. 4.  $\beta$ -oxidation activity in the gastrocnemius muscle from mice fed GTE. Muscles from mice fed the specified diets for 10 wk were homogenized, and oxidation activities for [ $^{14}$ C]-palmitic acid were measured as described in MATERIALS AND METHODS. Values are means  $\pm$  SE of 10 mice. \* $P < 0.05$ , vs. the Ex-control group.

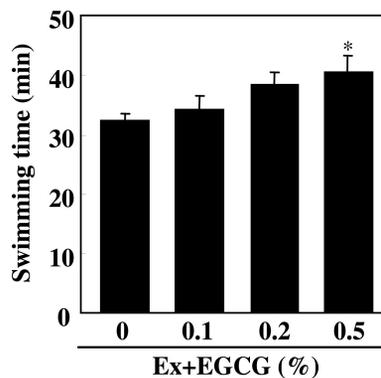


Fig. 5. Effects of epigallocatechin (EGCG) on endurance capacity for swimming. The swimming time to fatigue was measured at a flow rate of 7 l/min. Each point represents the mean swimming time of 10 mice. Bars represent means  $\pm$  SE. \* $P < 0.05$ , vs. the Ex-control group.

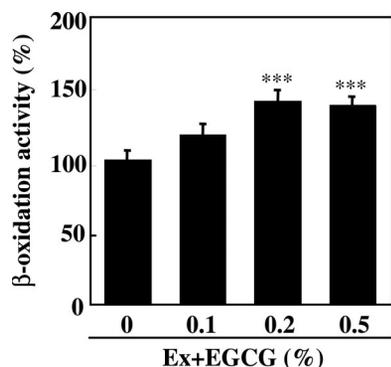


Fig. 6.  $\beta$ -oxidation activity in the muscle from mice fed EGCG. Muscles from mice fed the specified diets for 10 wk were homogenized, and oxidation activities for [ $^{14}$ C]-palmitic acid were measured as described in MATERIALS AND METHODS. Values are means  $\pm$  SE of 10 mice. \*\*\* $P$  < 0.001 vs. the Ex-control group.

of catecholamines (5, 9, 23) and the activity of adenylate cyclase, which catalyzes the formation of cAMP from ATP, thus enhancing lipolysis, fatty acid oxidation, and exercise performance (7, 9, 16, 49). However, the GTE used in this study contained less caffeine than the preparations used in previous studies. In addition, we observed no changes in plasma NEFA level under resting conditions (Table 2), suggesting that caffeine-stimulated lipolysis did not occur under these conditions. Thus our results overall suggest that the effects observed in this study are not attributable to caffeine. In particular, our findings that purified EGCG improved endurance capacity supports this conclusion.

Capsaicin has also been reported to improve endurance capacity in mice by transiently increasing fatty acid use as a result of catecholamine secretion (7, 9, 16, 19, 20, 49). However, because the effect of capsaicin is transient, it must be administered 2 h before swimming for the effect to be seen (19). In contrast, GTE in combination with regular exercise

seems to enhance basal lipid metabolism resulting in the stimulation of fatty acid use and therefore enhanced endurance capacity.

EGCG also improved the endurance capacity of mice, and this was accompanied by an upregulation of genes involved in  $\beta$ -oxidation in muscle and lipid metabolism, including FAT/CD36, (4, 14, 41). The capacity for increased fatty acid oxidation induced by exercise training has previously been reported (43) to be associated with genes such as FAT/CD36, which are involved in regulating fatty acid uptake across the plasma membrane. Although the extent of the changes in gene expression induced by EGCG was modest, considering the overall changes observed in this study, these changes in mRNA levels seem likely to contribute to individual energy metabolism and improvement in endurance capacity.

The precise molecular mechanism by which GTE stimulate fatty acid metabolism is unclear at present. In the last decade, it has become apparent that the expression of many fatty acid-metabolizing enzymes, including FAT/CD36, is transcriptionally regulated by PPARs (37). We confirmed that a series of catechins are not ligands for PPAR $\alpha$  and PPAR $\delta$  by using a transient transfection assay that combines a chimeric GAL4 DNA binding domain-PPAR ligand binding domain receptor and a reporter gene containing GAL4 binding sites (data not shown). It is possible that some posttranscriptional changes are implicated in the action of GTE during exercise. In addition, short but repeated exposures to increased plasma fatty acid concentrations during exercise may produce adaptive changes that stimulate lipid catabolism in the muscle.

Beneficial effects of physical exercise are well known; however, in some cases the muscular response to exercise may be considered injurious. For example, free radicals are produced under oxidative stress (1). Although the implications of oxidative stress for endurance capacity remain unclear, some compounds that have antioxidant activity have been reported to improve performance and prevent fatigue (45). Novelli et al.

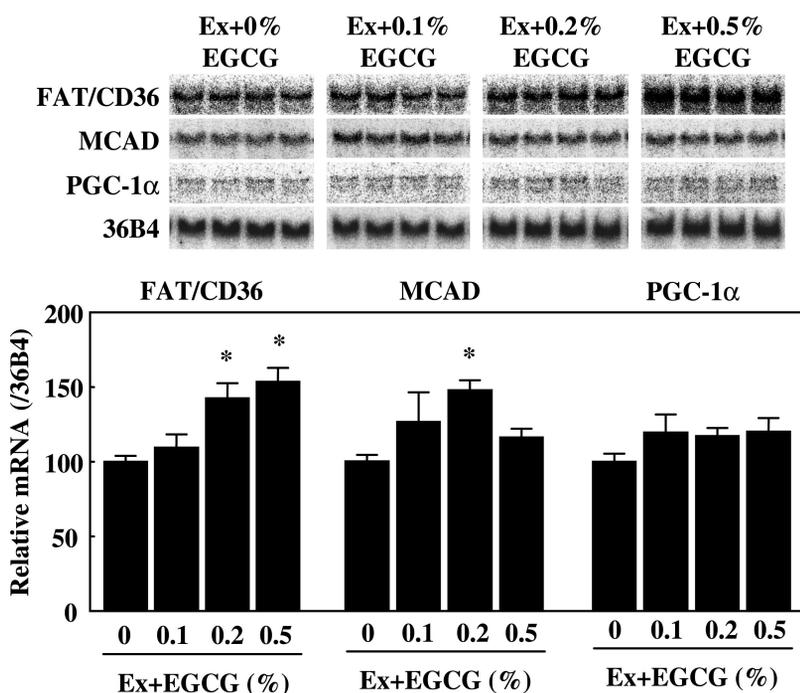


Fig. 7. mRNA expression in muscle from mice fed EGCG. Total RNA isolated from skeletal muscle was used in Northern blot analysis as described in MATERIALS AND METHODS. Amounts of FAT/CD36, medium-chain acyl-CoA dehydrogenase (MCAD), and PGC-1 $\alpha$  mRNA were normalized to 36B4 mRNA levels and expressed as percentages of the corresponding amounts in the Ex-control group. Values are means  $\pm$  SE of 10 mice. \* $P$  < 0.05, vs. the Ex-control group.

(32) reported that vitamin E prolonged resistance to muscle fatigue in mice. Other studies (2, 32, 34, 38, 45) carried out by using animal muscle preparations in vitro showed that the addition of antioxidants to the suspension medium delayed muscle fatigue. Therefore, it is possible that the antioxidant properties of tea catechins (35) mediate their effects on endurance capacity. The detailed molecular mechanism by which GTE enhances endurance capacity remains to be elucidated.

In summary, we have shown that GTE is beneficial for improving endurance capacity and that this effect is accompanied by a stimulation of lipid metabolism. Although the clinical efficacy of GTE has not yet been confirmed in human studies, our results suggest that GTE may be a useful tool for improving endurance capacity.

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