

Tea catechin ingestion combined with habitual exercise suppresses the aging-associated decline in physical performance in senescence-accelerated mice

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Murase T, Haramizu S, Ota N, Hase T. Tea catechin ingestion combined with habitual exercise suppresses the aging-associated decline in physical performance in senescence-accelerated mice. *Am J Physiol Regul Integr Comp Physiol* 295: R281–R289, 2008. First published May 14, 2008; doi:10.1152/ajpregu.00880.2007.—Catechins, which are abundant in green tea, possess a variety of biologic actions, and their clinical application has been extensively investigated. In this study, we examined the effects of tea catechins and regular exercise on the aging-associated decline in physical performance in senescence-accelerated prone mice (SAMP1) and age-matched senescence-accelerated resistant mice (SAMR1). The endurance capacity of SAMR1 mice, measured as the running time to exhaustion, tended to increase over the 8-wk experimental period, whereas that of SAMP1 mice decreased by 17%. On the other hand, the endurance capacity of SAMP1 mice fed 0.35% (wt/wt) catechins remained at the initial level and was significantly higher than that of SAMP1 mice not fed catechins. In SAMP1 mice fed catechins and given exercise, oxygen consumption was significantly increased, and there was an increase in skeletal muscle fatty acid β -oxidation. The mRNA levels of mitochondria-related molecules, such as peroxisome proliferator-activated receptor- γ coactivator-1, cytochrome *c* oxidase-II, III, and IV in skeletal muscle were also higher in SAMP1 mice given both catechins and exercise. Moreover, oxidative stress measured as thiobarbituric reactive substances was lower in SAMP1 groups fed catechins than in the SAMP1 control group. These results suggest that long-term intake of catechins, together with habitual exercise, is beneficial for suppressing the aging-related decline in physical performance and energy metabolism and that these effects are due, at least in part, to improved mitochondrial function in skeletal muscle.

energy metabolism; exercise; green tea; mitochondria; oxidative stress

MARKED DECLINE IN PHYSICAL performance with increasing age is a crucial problem in our aging society. As people age, skeletal muscle mass and strength decrease, fatty acid metabolism changes, physical activity declines, and body fat mass increases, which results in an increased incidence of lifestyle-related diseases, bed rest, and other morbidities (31). These aging-related alterations are assumed to be associated with changes in the expression and activity of muscular mitochondria-related molecules, such as cytochrome *c* oxidases (COXs), electron transfer chain enzymes, and peroxisome proliferator-activated receptor- γ coactivator-1 (PGC-1), which mediate mitochondrial biogenesis and oxidative metabolism and myosin heavy chain (2, 6, 31). In addition, these molecules are

thought to mediate exercise-induced transient or chronic metabolic responses.

Because some aging-related increases in obesity, lifestyle-related diseases, and physical impairment are caused by a lack of exercise and irregular eating habits, regular exercise and dietary improvements are recommended for their prevention (24, 36). Thus, many attempts have been made to maintain/improve physical performance and to suppress lifestyle-related diseases by nutritional and physical means (41, 42); however, their efficacy and practicality are limited and remain controversial (12, 17).

Green tea, one of the most popular beverages in the world, contains a series of polyphenols known as catechins. Tea catechins, consisting mainly of epigallocatechin gallate, epicatechin gallate, gallic catechin, and epigallocatechin, have a variety of properties, including antibacterial activity (40), anticarcinogenic activity (48), antidiabetic (22), and antiatherogenic (7, 25) effects. Moreover, tea catechins are proposed to be neuroprotective agents against progressive neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (45). Thus, there is a growing interest in the application of tea catechins for disease prevention and treatment (49).

We have been investigating the nutritional functions of tea catechins and demonstrated that long-term intake of tea catechins is beneficial for reducing diet-induced obesity in humans (30) and mice (26, 28, 39). More recently, we demonstrated that habitual intake of catechin-rich green tea extract (GTE) improves both swimming and running endurance capacity in normally-aging BALB/c mice (27, 29). In our previous study, swimming times to exhaustion in mice given habitual exercise were prolonged by 25% over a 10-wk period compared with the initial times. Swimming times of mice fed 0.5% GTE together with habitual exercise were 24% longer at week 10 than that of the group given exercise alone. The GTE-fed and exercised mice also exhibited increased activation of lipid catabolism in skeletal muscle. Thus, the endurance-improving effects of GTE were thought to be mediated, at least in part, by increased metabolic capacity and utilization of fatty acid as a source of energy in skeletal muscle.

Senescence-accelerated mice (SAM) are inbred strains of AKR mice that are murine models of accelerated aging (43). SAM comprise two strains: senescence-accelerated prone mice (SAMP) that show accelerated senescence, loss of behavioral activity, impaired physiological function, higher oxidative stress, and a shorter lifespan; and senescence-accelerated re-

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sistant mice (SAMR), the corresponding control mouse strain that ages normally (8, 14, 35, 37). SAM strains display aging-associated characteristics similar to those observed in humans and are therefore widely used in aging research.

Based on the ability of catechins to stimulate muscle lipid metabolism and their antioxidative properties, we hypothesized that catechin intake might counteract aging-associated changes in physical performance and metabolic status by preventing aging-induced impairment of mitochondrial function and decreased energy production. To test this, we examined the effects of tea catechin ingestion on the aging-associated decline in physical function, such as endurance capacity and muscular energy metabolism in SAM.

MATERIALS AND METHODS

Tea catechins. Tea catechins were prepared and analyzed as described previously (27). Total catechin content was 81% (the sum of all catechin types). The catechins comprised epigallocatechin gallate (41%), epigallocatechin (23%), epicatechin gallate (12%), epicatechin (9%), gallic catechin (7%), gallic catechin gallate (4%), and others (4%).

Animals and diets. Male SAMP1 and SAMR1 mice (13-wk-old) were obtained from SLC Japan (Hamamatsu, Japan) and maintained at $23 \pm 2^\circ\text{C}$ under a 12:12-h light-dark cycle (lights on from 07:00 to 19:00). The mice were fed laboratory chow (CE-2; CLEA Japan, Tokyo, Japan) and had free access to drinking water for 5 wk to stabilize their metabolic condition. At 18 wk of age, the mice were trained to run on a treadmill to accustom them to treadmill running and to eliminate self-injurious mice, and then their initial endurance capacity was measured at *week 19*, as described below. SAMP1 mice were divided into four groups (control, catechin, exercise, and catechin+exercise groups, $n = 8$ each). The SAMR1 mice were divided into two groups (control and exercise groups, $n = 8$ each), all of which were allowed unlimited access to water and given a synthetic diet containing 10% (wt/wt) fat, 20% casein, 55.5% potato starch, 8.1% cellulose, 2.2% vitamins, 0.2% methionine, and 4% minerals. The diet of the catechin groups was supplemented with 0.35% tea catechins. The animals were housed individually and fed their respective diets for 10 wk in plastic cages with nest boxes (Shepherd Specialty Papers, Watertown, TN) to reduce stress. During the experiments, the animals were cared for in accordance with the American Physiological Society's "Guiding Principles for the Care and Use of Animals." This study was approved by the Animal Care Committee of Kao Tochigi Institute, Japan.

Exercise and evaluation of endurance. A 10-lane motorized rodent treadmill (Muromachi Kikai, Tokyo, Japan) was used to determine running endurance capacity. At 18 wk of age, the mice were run on the treadmill at an inclination of 7 degrees, and underwent the following 5-day running program: *day 1*: 10 m/min for 15 min, and 15 m/min for 15 min; *day 2*: 10 m/min for 5 min, and 15 m/min for 25 min; *day 3*: 10 m/min for 5 min, 15 m/min for 15 min, and 20 m/min for 10 min; *day 4*: 10 m/min for 5 min, 15 m/min for 10 min, and 20 m/min for 15 min; and *day 5*: 10 m/min for 5 min, 15 m/min for 5 min, and 20 m/min for 20 min.

At 19 wk of age, initial running times to exhaustion were measured according to the following program: 10 m/min for 5 min, 15 m/min for 5 min, 20 m/min for 60 min, 22 m/min for 60 min, 24 m/min for 60 min, and 26 m/min for 60 min.

To reduce the inherent variation in running capacity, 32 of 60 SAMP1 and 16 of 30 SAMR1 mice were selected by eliminating those mice whose running time was 30% longer or shorter than the average. Average running time and body weight were adjusted between the groups.

At 20 wk of age, the mice were divided into groups as described above, and the feeding and exercise programs began. During the

experimental period, they were exercised on a treadmill three times a week at a speed of 15 m/min for 30 min (with the exception of the nonexercise group). Eight weeks after the start of the experiment, their running endurance capacity was measured. At the 10th week, the mice were killed and dissected 2 days after the final running training under nonfasting and nonexercised conditions.

Food intake. Food intake was measured for each mouse by weighing the food that remained in the cage every 2 or 3 days. Feed efficiency was calculated as: body weight gain per mouse (kg)/kcal of total food consumed per mouse. The energy values for each diet were calculated from the macronutrient composition using values of 4 kcal/g, 4 kcal/g, and 9 kcal/g for carbohydrate, protein, and oil, respectively.

Body and tissue weight. Body weight was measured weekly. On the last day of the experiment, fat pad weight (retroperitoneal, epididymal, and perirenal fat), skeletal muscle weight (gastrocnemius, soleus, and plantaris muscle), and liver weight were determined. The ratio of each tissue to total body weight was calculated.

Indirect calorimetry. Energy metabolism was analyzed in the 9th week of the experiment using an individual open-circuit indirect calorimeter (Oxymax; Columbus Instruments, Columbus, OH). The mice were allowed free access to the control diet, and oxygen consumption ($\dot{V}\text{O}_2$) and carbon dioxide production ($\dot{V}\text{CO}_2$) were monitored for 24 h. During this time, data for each chamber were collected every 18 min with a settling time of 30 s, a measurement time of 90 s, and room air as reference. Respiratory quotient was calculated from the $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ values.

Measurement of fatty acid β -oxidation activity. Fatty acid β -oxidation activity was measured using [$1\text{-}^{14}\text{C}$]-palmitic acid as the substrate as described previously (29). Frozen gastrocnemius or soleus muscle was thawed and homogenized on ice using a polytron homogenizer (Microtech, Chiba, Japan) in 250 mM sucrose and 1 mM EDTA in 10 mM HEPES (pH 7.2). Subcellular debris was removed by centrifugation at 600 g for 5 min, and the supernatant was used for the assay.

Biochemical analysis. Plasma triglyceride, nonesterified fatty acid (NEFA), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total cholesterol, glucose, and HDL-cholesterol concentrations were measured using the L-type TG-H, NEFA-HA, L-type GOT J2, L-type GPT J2, L-type CHO-H, L-type Glu2, and L-type HDL-C assay kits (Wako, Osaka, Japan), respectively. Plasma insulin and leptin levels were measured using mouse insulin and leptin enzyme immunoassay kits (Morinaga, Yokohama, Japan). Adiponectin level was analyzed with a mouse adiponectin enzyme-linked immunosorbent assay kit (Otsuka, Tokyo, Japan).

RNA extraction and real-time PCR. On the final day of the experiment, the gastrocnemius muscles were dissected from each animal and frozen in liquid nitrogen for subsequent RNA isolation. Total RNA was isolated with Isogen (Wako) according to the manufacturer's instructions. Reverse transcription was performed with a SuperScript first-strand synthesis system (Invitrogen, Carlsbad, CA). Transcript levels in the muscle were examined by real-time PCR with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). Real-time PCR was performed with an ABI PRISM model 7000 sequence detector (Applied Biosystems) using the following protocol: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. mRNA levels were calculated relative to those of 36B4 mRNA, and the normalized values were expressed as percentages, with the value for the SAMP1 control (SAMP1-Con) mice set at 100%. The primers used are shown in Table 1.

Plasma thiobarbituric reactive substances. Plasma thiobarbituric reactive substances (TBARS) were measured with an OXI-TEK TBARS assay kit (Alexis, Lausanne, Switzerland) according to the manufacturer's instructions. Protein concentrations were determined with a BCA protein assay kit (Pierce, Rockford, IL).

Table 1. Sequences of PCR primers

Gene	Accession No.	Primer Sequences	Position
COX-I	X57780	F: TCATACCTCAAAGCAACGAAGC R: GGCCCCCAATTCAGGTTAAG	876–897 957–976
COX-II	AF377830	F: CATGAGCAGTCCCCTCCCTA R: TTGGCCATAGAATAACCGTGGT	488–507 567–588
COX-III	NC_005089	F: TCTTCATGGCTACTGGATTCCA R: ATCATGCTGCGGCTTCAAAT	9196–9217 9308–9327
COX-IV	NM_053091	F: AGCTGAGCCAAGCAGAGAAG R: AATCACCAGAGCCGTGAATC	276–295 396–415
Cytochrome <i>c</i>	NM_007808	F: CCAAACTCTCCACGGTCTGTTC R: ATCAGGGTATCCTCTCCCCAG	159–179 245–265
ATP synthase	NM_016774	F: AGGGGCACCAATCAAATATCC R: GCCTCAGCATGAATAGGAGCAA	404–424 508–529
Citrate synthase	NM_026444	F: TGCCCAACACAGCCATTTG R: CTGACACGTCTTTGCCAACTT	899–917 1039–1059
Mitochondrial creatine kinase	NM_009897	F: TGCCACAGGCAGCGTCTTTGA R: TGACTTGCCAAGTCGATCCAA	1295–1315 1326–1346
Malate dehydrogenase 2	NM_008617	F: CCAGGAATGACACGGGATGAC R: GGTGGAATGATGCAAAACCA	403–423 494–514
HSP72	NM_010479	F: AGAACGGGCTCGAATCCTTGA R: AGGAGATGACCTCCTGGCACTT	1815–1835 1916–1937
PGC-1 α	NM_008904	F: CCGAGAATTCATGGAGCAAT R: TTTCTGTGGGTTTGGTGTGA	642–661 751–770
PGC-1 β	NM_133249	F: ACGGTTTATCACCTTCCGGT R: ATAGCTCAGGTGAAGGAGGG	2865–2885 2945–2965

COX, cytochrome *c* oxidase; HSP72, heat shock protein 72; PGC, proliferator-activated receptor- γ coactivator.

Statistical analysis. All values are presented as the means $\pm$ SE. Comparisons of data were made using a one-way ANOVA. When ANOVA indicated significant differences, each group was compared with the others by Fisher's protected least significant difference test (for comparison among SAMP1 groups) or Dunnett's test (for comparison between SAMP1-Con and SAMR1 groups). Statistical analysis of endurance was conducted using paired *t*-tests. Results with values of $P < 0.05$ were considered statistically significant. Statistical analyses were made using StatView software (SAS Institute Cary, NC).

RESULTS

Body weight and tissue weights. The final body weight of the SAMP1-Con mice was higher than that of the SAMR1 control mice (SAMR1-Con; Table 2), indicating that SAMP1 mice are prone to obesity. The body weights of the mice fed catechins or given exercise were lower, and this effect was increased by combining the two treatments. The difference between SAMP1-Con and SAMP1 catechin+exercise (SAMP1-CatEx) groups at each time point was significant during weeks 4 to 10 of the experiment (data not shown). The body weight of mice fed catechins alone were also significantly lower than that of control mice during weeks 4 to 8 (data not shown). Feed efficiency, calculated by body weight gain and energy intake, was significantly higher in SAMP1 mice than in SAMR1 mice, but significantly lower in the SAMP1-CatEx group (Table 2). To examine the effects of catechins on intra-abdominal fat and skeletal muscle mass, we measured the distribution of these tissues. After 10 wk, total fat weight was significantly higher in the SAMP1-Con group compared with the SAMR1-Con group, but the differences among SAMP1 groups were not significant. Perirenal fat weight was significantly decreased by both catechin intake and exercise. On the other hand, total muscle weight was significantly lower in the SAMP1 groups. The relative ratio of total muscle was slightly, but significantly,

elevated in the SAMP1-CatEx group. The liver weight of SAMP1-Con mice was significantly elevated and was reduced by both catechin intake and the combination of catechin intake and exercise.

Blood analysis. Blood was collected under nonfasting and nonexercised conditions, and plasma insulin and leptin levels were 234% and 45% higher, respectively, in the SAMP1-Con group than in the SAMR1-Con group (Table 3), indicating the development of senescence-associated hyperinsulinemia and hyperleptinemia. Plasma insulin concentrations were significantly reduced in the SAMP1 mice fed catechins (SAMP1-Cat and SAMP1-CatEx); however, we detected no significant difference in leptin levels. Plasma adiponectin was 38% lower in the SAMP1-Con than in the SAMR1-Con group, and 32% and 43% higher in the SAMP1-Cat and SAMP1-CatEx groups, respectively, than in the SAMP1-Con. Plasma glucose levels tended to be low in catechin-fed groups, but the differences were not significant. Liver function, as represented by GOT and GPT activities, deteriorated in SAMP1 mice and this effect was significantly blocked by catechin consumption. No significant differences were observed in NEFA, total cholesterol, and HDL-cholesterol.

Running endurance capacity. Figure 1 shows the running times to exhaustion of the mice before and after 8 wk of the experiment. The initial running time of SAMR1 mice was 19 min longer than that of age-matched SAMP1-Con mice, but the difference was not significant. The running times of SAMR1 mice (SAMR1-Ex) tended to increase over the 8 wk, but again the effect was not significant. On the other hand, the running time of SAMP1 mice (SAMP1-Ex) decreased significantly (by 21 min) over the 8-wk period, indicating an aging-related decline in endurance capacity. By contrast, the running time of the mice fed catechins (SAMP1-CatEx) remained at the initial level, and was significantly higher than that of SAMP1-Ex

Table 2. Energy intake, body weight, tissue weight, and relative tissue weight

	SAMR1		SAMP1			
	Con	Ex	Con	Cat	Ex	CatEx
Energy intake, kcal	865.1±8.6	859.0±7.7*	902.4±15.5 ^a	895.0±21.2 ^{ab}	983.1±16.7 ^b	898.5±15.0 ^{ab}
Feed efficiency, kg/kcal	7.1±0.6*	5.5±1.1*	15.8±1.6 ^a	12.6±1.2 ^{ab}	12.8±1.1 ^{ab}	10.6±1.7 ^b
Initial body weight, g	32.14±0.51	32.07±0.83	32.43±1.40	32.43±1.07	32.59±1.49	32.43±1.14
Final body weight, g	38.30±0.71*	37.12±1.20*	46.69±1.57 ^a	43.82±1.78 ^{ab}	45.18±1.42 ^{ab}	42.00±1.27 ^b
Gastrocnemius, mg	282.9±8.0*	290.4±3.6*	243.5±8.4	235.0±6.1	246.4±9.5	242.9±4.5
Soleus, mg	18.8±1.0*	17.3±0.9*	14.9±0.9	14.0±0.9	15.0±1.1	15.8±0.4
Plantaris, mg	34.1±0.8*	34.7±1.1*	28.3±1.0	28.1±0.7	29.5±1.3	28.6±0.8
Total muscle, mg#	335.8±8.7*	342.4±4.1*	286.6±10.0	277.1±6.0	290.9±11.3	287.3±5.4
Liver, g	1.80±0.05*	1.78±0.09*	2.25±0.17 ^a	1.79±0.11 ^b	1.90±0.17 ^{ab}	1.64±0.07 ^b
Epididymal fat, g	1.75±0.14*	1.43±0.14*	2.35±0.09	2.31±0.17	2.25±0.12	2.14±0.08
Perirenal fat, g	0.21±0.02*	0.18±0.01*	0.40±0.02 ^a	0.28±0.03 ^b	0.30±0.02 ^b	0.25±0.02 ^b
Retroperitoneal fat, g	0.46±0.02	0.40±0.04	0.42±0.01	0.45±0.02	0.41±0.02	0.40±0.02
Total fat, g##	2.42±0.17*	2.01±0.18*	3.17±0.09	3.04±0.21	2.96±0.14	2.78±0.10
Gastrocnemius, %	0.739±0.015*	0.786±0.021*	0.522±0.005 ^a	0.540±0.019 ^{ab}	0.546±0.015 ^{ab}	0.581±0.017 ^b
Soleus, %	0.049±0.003*	0.047±0.002*	0.032±0.002 ^a	0.032±0.003 ^{ab}	0.033±0.002 ^{ab}	0.038±0.001 ^b
Plantaris, %	0.089±0.002*	0.094±0.004*	0.061±0.001 ^a	0.065±0.002 ^{ab}	0.065±0.002 ^{ab}	0.068±0.002 ^b
Total muscle, %#	0.877±0.017*	0.927±0.025*	0.614±0.006 ^a	0.637±0.021 ^{ab}	0.644±0.017 ^{ab}	0.687±0.019 ^b
Liver, %	4.68±0.09	4.77±0.11	4.77±0.23 ^a	4.07±0.14 ^b	4.16±0.27 ^b	3.90±0.05 ^b
Epididymal fat, %	4.54±0.29	3.81±0.24*	5.07±0.24	5.24±0.28	5.05±0.38	5.09±0.16
Perirenal fat, %	0.55±0.05*	0.48±0.02*	0.85±0.05 ^a	0.62±0.05 ^b	0.65±0.03 ^b	0.58±0.03 ^b
Retroperitoneal fat, %	1.19±0.05	1.08±0.08*	0.92±0.01	1.03±0.04	0.92±0.07	0.96±0.07
Total fat, %##	6.28±0.34	5.37±0.30*	6.84±0.26	6.89±0.31	6.63±0.45	6.64±0.21

Values are the means ± SE of 8 mice. SAMR, senescence-accelerated resistant mice; SAMP, senescence-accelerated prone mice; Con, control; Ex, exercise; Cat, catechins. #Sum of gastrocnemius, soleus, and plantaris muscles; ##sum of epididymal, perirenal, and retroperitoneal fat. Relative tissue weight was presented as the ratio of each tissue to total body weight. Differences between SAMP1-Con and the 2 SAMR1 groups were compared by Dunnett's test: **P* < 0.05, vs. the SAMP1-Con group. Differences among the 4 SAMP1-groups were compared by Fisher's test. ^{a,b}Statistically significant differences are denoted by different letters.

mice. These results suggest that catechin consumption effectively suppressed the decline in physical performance.

Whole body energy metabolism. Metabolic differences between SAMP1 and SAMR1 mice, and the effect of catechins on energy metabolism were examined by indirect calorimetry. Oxygen consumption was markedly different between SAMP1 and SAMR1 mice (Fig. 2). The oxygen consumption of SAMP1-Con mice was significantly lower than that of SAMR1-Con mice and was the same in the SAMP1-CatEx group as in the SAMR1-Con group.

Fatty acid β-oxidation activity in the skeletal muscle. Fatty acid β-oxidation activity in gastrocnemius and soleus muscles was significantly lower in SAMP1 mice than SAMR1 mice

(Fig. 3). The β-oxidation activity in the muscles was significantly increased by catechin intake and exercise.

mRNA expression levels in skeletal muscle. To determine the basis of senescence-associated changes in physical performance, energy metabolism, and how these two parameters were affected by catechins and exercise, we analyzed various mRNAs in gastrocnemius muscle using quantitative RT-PCR. Marked changes were observed in mitochondrial electron transport chain components (Fig. 4). The mRNA levels of cytochrome C, cytochrome C oxidase-II, -III, and -IV in SAMP1-Con were 19%, 29%, 28%, and 37% lower, respectively, than in the SAMR1-Con. Expression of these mRNAs in the SAMP1-CatEx group was 16%, 21%, 20%, and 29%

Table 3. Plasma analysis

	SAMR1		SAMP1			
	Con	Ex	Con	Cat	Ex	CatEx
Glucose, mg/dl	186.3±11.2*	215.9±13.4	292.8±37.2	211.8±24.8	274.1±21.9	250.5±32.7
NEFA, meq/l	1.72±0.09	1.41±0.14	1.57±0.09 ^a	1.53±0.11 ^{ab}	1.29±0.08 ^{ab}	1.21±0.09 ^b
Triglyceride, mg/dl	207.3±37.1	132.8±17.4*	243.9±29.2 ^a	213.4±5.9 ^{ab}	170.9±13.4 ^b	189.1±14.0 ^b
Total cholesterol, mg/dl	215.1±8.7	233.7±18.7	215.1±13.9	203.2±7.8	200.1±12.6	204.6±5.2
HDL-cholesterol, mg/dl	155.8±5.4	164.7±8.5	143.9±7.5	137.0±5.1	137.5±7.1	138.9±3.2
GOT, IU/l	32.5±1.6*	34.3±1.9*	53.2±3.0 ^a	42.2±2.2 ^b	41.5±1.5 ^b	40.1±2.5 ^b
GPT, IU/l	19.1±1.7*	22.7±4.1*	42.8±4.4 ^a	27.8±2.4 ^b	32.5±2.2 ^b	26.1±2.4 ^b
Insulin, ng/ml	3.5±0.6*	4.3±1.0*	11.7±1.3 ^a	7.0±1.2 ^b	10.5±1.1 ^{ab}	7.7±1.1 ^b
Adiponectin, μg/ml	15.3±0.9*	14.6±0.6*	9.5±0.8 ^a	12.5±0.6 ^b	11.6±0.8 ^b	13.6±0.7 ^b
Leptin, ng/ml	13.6±1.3*	10.9±1.8*	19.7±0.7	18.5±1.5	19.1±0.5	17.8±1.1
TBARS, nmol/ml	0.64±0.02*	0.77±0.04*	1.25±0.12 ^a	0.92±0.09 ^b	1.00±0.10 ^{ab}	0.91±0.08 ^b

Values are the means ± SE of 8 mice. NEFA, nonesterified fatty acid; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; TBARS, thiobarbituric reactive substances. Differences between SAMP1-Con and the 2 SAMR1 groups were compared by Dunnett's test: **P* < 0.05 vs. the SAMP1-Con group. Differences among the 4 SAMP1-groups were compared by Fisher's test. ^{a,b}Statistically significant differences are denoted by different letters.

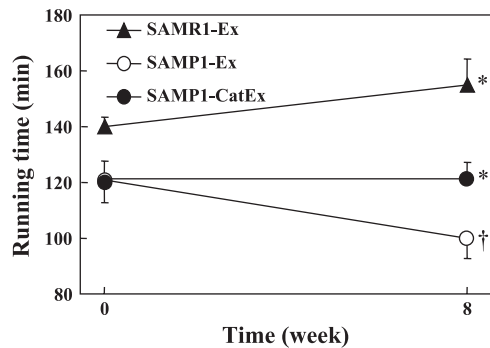


Fig. 1. Effects of catechins on running endurance in senescence-accelerated prone mice (SAMP1) and senescence-accelerated resistant mice (SAMR1). Running time to fatigue was measured. Cat, catechins; Ex, exercise. Values represent the means $\pm$ SE of 8 mice. * P < 0.05 vs. SAMP1-Con group at 8 wk (Dunnett's test). † P < 0.05 vs. initial value (t -test).

higher than in the SAMP1-Con group. On the other hand, transcripts of the tricarboxylic acid cycle enzymes, citrate synthase, and malate dehydrogenase, were also low in the SAMP1-Con, but none of the treatments had any effect on the levels of these enzymes. As PGC-1 is reported to be a crucial determinant of mitochondrial biogenesis and energy metabolism (10), we examined the levels of PGC-1 α and -1 β mRNAs. The expression levels of PGC-1 α and -1 β mRNA were slightly, but significantly, elevated in the SAMP1-CatEx group. Heat shock protein 72, a stress- and exercise-inducible protein, is thought to play an important role in preventing muscle damage and maintaining protein synthesis in skeletal muscle (9, 32). Heat shock protein 72 mRNA was increased by exercise and was 23% higher in the SAMP1-CatEx group than in the SAMP1-Con group.

Antioxidative activity. We measured the level of lipid peroxidation products as TBARS. SAMP1-Con mice had a higher plasma TBARS level than SAMR1 mice, implying an aging-associated increase in oxidative stress (Table 3). TBARS content was significantly reduced in both the SAMP1-Cat and SAMP1-CatEx groups compared with SAMP1-Con mice.

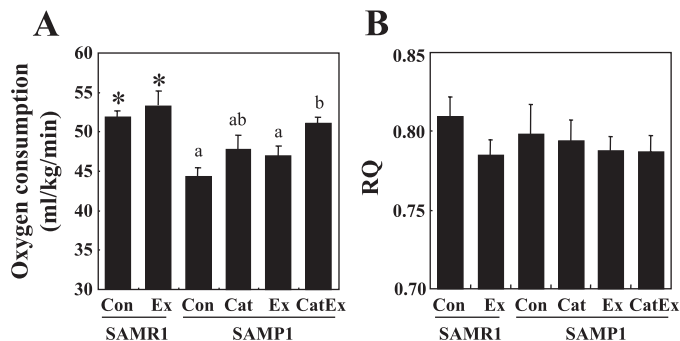


Fig. 2. Effect of catechins and habitual exercise on whole body energy metabolism. Oxygen consumption (A) and CO_2 exhalation were measured after 9 wk, and respiratory quotient (RQ; B) was calculated. Values represent the means $\pm$ SE of 8 mice. Differences between SAMP1-Con and both SAMR1 groups were compared by Dunnett's test: * P < 0.05 vs. the SAMP1-Con group. Con, control. Differences among the 4 SAMP1-groups were compared by Fisher's test. ^{a,b}Statistically significant differences are denoted by different letters.

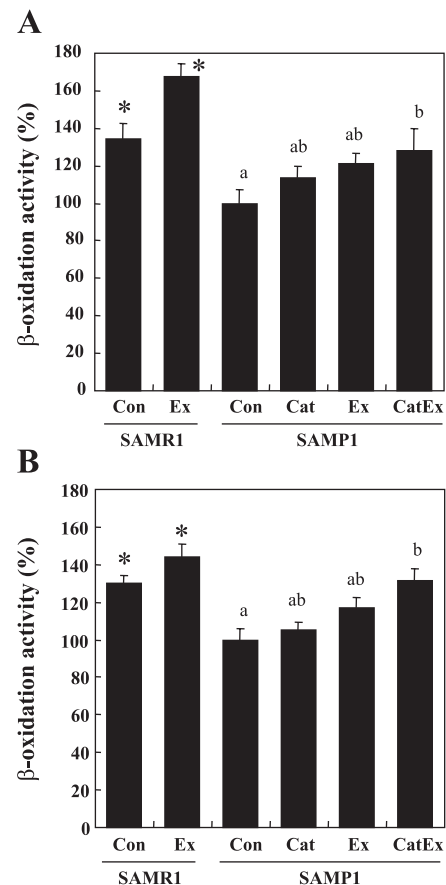


Fig. 3. Effect of tea catechins and habitual exercise on fatty acid β -oxidation activity in gastrocnemius (A) and soleus (B) muscles. Gastrocnemius or soleus muscles of mice were homogenized, and [1- ^{14}C]-palmitic acid oxidation activity was measured as described in MATERIALS AND METHODS. Enzyme activity is expressed as a percentage, taking levels of SAMP1-Con as 100%. Values represent the means $\pm$ SE of 8 mice. Differences between SAMP1-Con and both SAMR1 groups were compared by Dunnett's test: * P < 0.05 vs. the SAMP1-Con group. Differences among the 4 SAMP1-groups were compared by Fisher's test. ^{a,b}Statistically significant differences are denoted by different letters.

DISCUSSION

In this study we examined the effects of tea catechins and exercise on physical performance in SAM and demonstrated that dietary supplementation with tea catechins markedly suppressed the aging-related decline in endurance capacity, accompanied by increased energy metabolism. We suggest that the preservation of energy metabolism by tea catechins in combination with exercise is mediated, at least in part, by preventing mitochondrial dysfunction in skeletal muscle.

Consistent with previous reports (8, 14, 35, 37, 43), SAMP1 mice exhibited many characteristics of aging compared with age-matched SAMR1 mice. The endurance capacity of SAMR1 mice tended to increase over the 8 wk of the experimental period, whereas that of SAMP1 mice decreased (Fig. 1). The difference between SAMP1 and SAMR1 mice seems to be partly attributable to the difference in energy metabolism. Muscular fatty acid β -oxidation activity and expression of mRNA related to energy metabolism were lower in SAMP1 mice than in SAMR1 mice (Fig. 3, 4), which might be associated with lower energy production from lipids during exer-

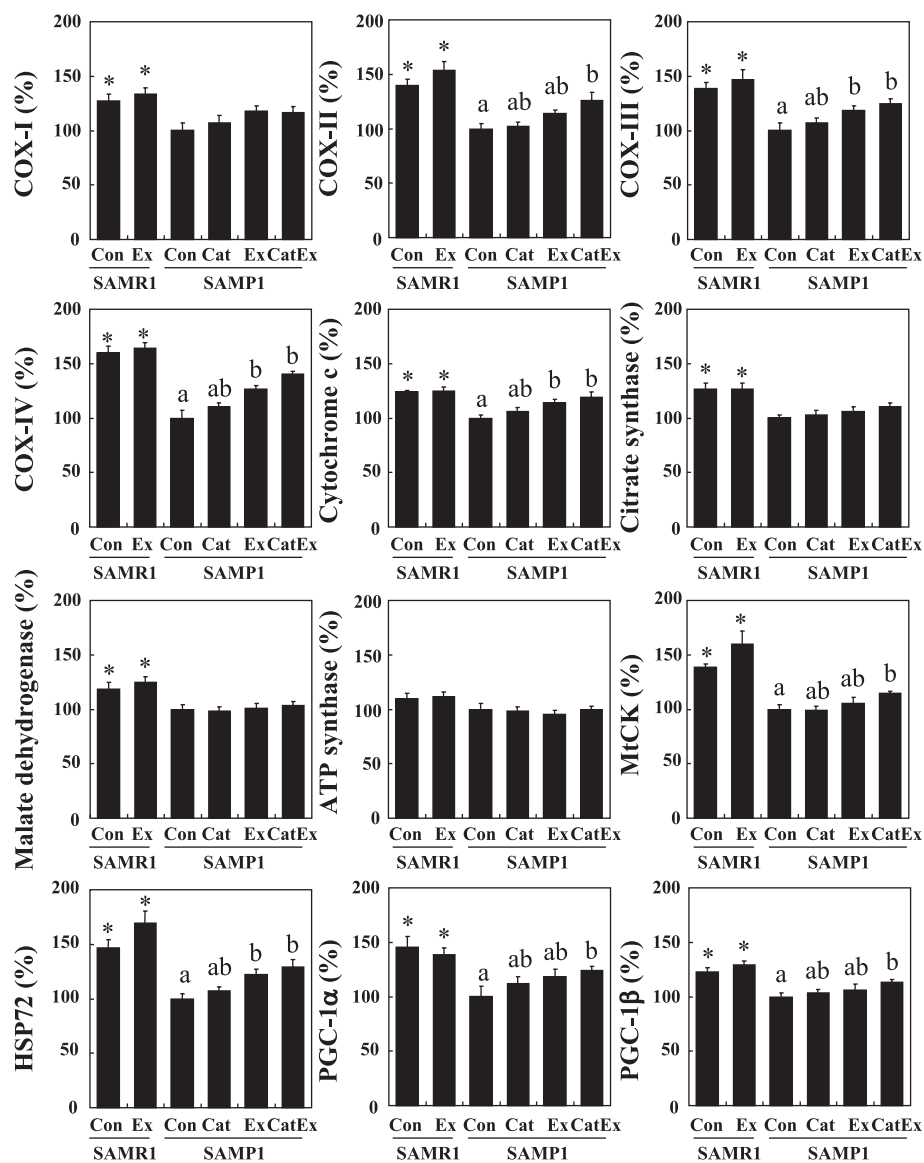


Fig. 4. mRNA expression in skeletal muscle. Total RNA isolated from gastrocnemius muscle was subjected to real-time RT-PCR. The amount of each mRNA was normalized to 36B4 mRNA levels and is expressed as a percentage of the corresponding amount in the SAMP1-Con group. COX, cytochrome *c* oxidase; MtCK, mitochondrial creatine kinase; HSP72, heat shock protein 72; PGC, proliferator-activated receptor- γ coactivator. Values are the means $\pm$ SE of 8 mice. Differences between SAMP1-Con and both SAMR1 groups were compared by Dunnett's test: * $P < 0.05$ vs. the SAMP1-Con group. Differences among the 4 SAMP1-groups were compared by Fisher's test. ^{a,b}Statistically significant differences are denoted by different letters.

cise, leading to a lower running endurance. In addition, SAMP1 mice appeared to display senescence-associated mitochondrial dysfunction. These results indicate that the decline in mitochondrial function during aging greatly influences the physical performance as well as the whole body energy metabolism in SAMP1 mice.

Analysis of the effects of tea catechins on aging-associated changes in physical performance in SAMP1 mice indicated that tea catechins suppressed the decline in endurance capacity and fatty acid β -oxidation activity and the expression of mRNA related to energy metabolism in muscle. During aerobic exercise, skeletal muscle mainly relies on carbohydrate and fatty acids for energy (15, 16). Enhanced utilization of fatty acids is thought to reduce carbohydrate utilization and to result in greater endurance capacity. Muscular lipid oxidation was maintained at a high rate in the SAMP1-CatEx group (Fig. 3), indicating that the maintenance of a high capacity for lipid utilization may contribute to the improvement of physical performance brought about by tea catechins.

In SAMP1 mice fed catechins and given exercise (SAMP1-CatEx), COXs and cytochrome *c* mRNAs were higher than in the SAMP1-Con (Fig. 4). The corresponding proteins are required for mitochondrial electron transport and decrease with age, resulting in reduced ATP production (2, 31). Therefore the improved mitochondrial function induced by catechins and exercise may contribute to increase energy expenditure and improve physical performance. Recent studies revealed that PGC-1 is an inducible regulator of mitochondrial biogenesis and function, energy production, and muscle fiber type (10, 11). The expression levels of PGC-1 α and -1 β were paralleled by oxygen consumption and the mRNA levels of mitochondrial components (Figs. 2 and 4), supporting the important role of PGC-1 in the regulation of mitochondrial function and whole body energy metabolism. Recently, Wisloff et al. (47) characterized artificially selected rats with low vs. high endurance capacity, and demonstrated that impaired regulation of mitochondrial biogenesis and oxidative pathways in skeletal muscle is associated with decreased aerobic capacity and increased

cardiovascular risk. Expression of PGC-1 α and COX is markedly higher in high-endurance capacity rats than in low-endurance capacity rats. Furthermore, resveratrol, a natural polyphenolic compound found in grape skin, improves mitochondrial function, increases aerobic capacity, and promotes the survival of mice by activating SIRT1 and PGC-1 α (3, 19). These results support our finding that the improved mitochondrial function by catechins and exercise plays an essential role in increased physical performance. In the present study, we used type-IIb myosin heavy chain-rich gastrocnemius muscle for the analysis of mRNA expression due to the limited availability of soleus muscle. To clarify the precise molecular mechanism, further studies on the regulation of mRNA expression in another skeletal muscle, including type-I myosin heavy chain-rich soleus, is required.

Oxidative stress increases during aging and is closely associated with mitochondrial dysfunction (38). The higher oxidative stress observed in SAMP mice is considered to be a cause of the accelerated senescence and aging-related changes in physical function (14). Oxidative stress measured as TBARS was lower in both SAMP1-Cat and SAMP1-CatEx groups than in the SAMP1-Con (Table 3), suggesting that the reduction of oxidative stress by catechins contributes, at least in part, to the maintenance of mitochondrial function and, hence, physical function. These effects of catechins are mostly attributable to their antioxidative properties (20, 34).

The higher rate of whole body energy expenditure, muscular β -oxidation activity, and mRNA expression involved in mitochondrial energy metabolism in the SAMP1-CatEx group also contributes to a reduction in aging-related body weight gain. In addition, the stimulating effect of catechins on hepatic lipid catabolism may contribute to the reduction in body weight in groups fed catechins (26). With the increase in body fat mass, various metabolic changes are induced. Over the past decade, it has become evident that adipocyte-derived hormones have a crucial role in energy homeostasis and the development of obesity and lifestyle-related diseases (18, 23). Adiponectin, in particular, whose expression decreases with the development of obesity, stimulates energy metabolism, improves insulin resistance, and prevents atherosclerosis. The plasma adiponectin concentration in SAMP1-Con mice was significantly lower than that in the SAMR1-Con, but was significantly increased by catechin intake and exercise (Table 3). The higher level of adiponectin in the SAMP1-CatEx group may have contributed to their greater energy expenditure. The sustained level of adiponectin in SAMP1-CatEx raises the possibility that catechin intake effectively prevents or improves lifestyle-related diseases such as diabetes and atherosclerosis; however, because these findings concerning adiponectin are roughly paralleled by changes in body weight, it is difficult to determine whether the changes in adiponectin concentration are a primary cause or a secondary consequence of the reduced body fat accumulation. We cannot, however, rule out the possibility that the difference in oxygen consumption observed in expired gas analysis was a result of a difference in the activity of the mice.

Our overall results indicated that significant physical and metabolic effects are induced by concomitant intake of catechins with habitual exercise. Treatment with catechins or exercise alone exerts similar effects in some aspects, but the changes in the parameters varied. For example, exercise significantly upregulated COXs and heat shock protein 72 mRNA

expression, but catechins alone did not (Fig. 4). Exercise also had a significant plasma triglyceride-lowering effect (Table 3). A role for AMP-activated protein kinase (AMPK) has been suggested in the regulation of mitochondrial function and energy metabolism (1, 21, 50). AMPK is activated by exercise and promotes fatty acid oxidation via a reduction in malonyl-CoA. AMPK is also associated with increased expression of a number of transcription factors involved in mitochondrial biogenesis and enzyme content (4, 46). Therefore, some biologic alterations observed in the exercised group might be explained by effects on AMPK pathway, which links the acute or chronic metabolic response to exercise.

A significant endurance-maintaining effect was observed in SAMP1 mice given both catechins and exercise compared with mice given exercise alone (Fig. 1), indicating that catechins have a clear effect on physical performance. On the other hand, it is not clear whether catechin intake alone improves endurance capacity. Based on the results of the present study and a previous study (28), the effect of catechins alone might be limited because catechin intake alone had no marked effect on muscle metabolism, including fatty acid β -oxidation activity and mRNA expression. Rather, catechins seem to exert their main effects by stimulating hepatic lipid catabolism (26, 28, 39), which leads to reduced liver and abdominal fat weight and a lower plasma insulin level (Table 2, 3).

Concomitant catechin ingestion and habitual exercise significantly improved energy metabolism and physical performance, suggesting some interaction between catechins and exercise. Catecholamine release, which promotes lipid mobilization and thermogenesis via β_3 -adrenoreceptors, increases during exercise (13, 33, 44). As catechins are reported to inhibit catechol-*O*-methyltransferase, the enzyme that degrades catecholamines (5), it is possible that catechins augment the sympathetic stimulation of energy metabolism, especially in combination with exercise. In addition, the antioxidative properties of catechins may also contribute to the combined effects of catechins and exercise by diminishing the oxidative stress brought about by aerobic exercise (Table 3) (20, 34). The interaction between catechins and exercise, and the precise mechanisms underlying the energy metabolism-stimulating effect and resultant endurance-maintaining effect of catechins in combination with exercise require further investigation.

The clinical efficacy of catechins against aging-related changes in humans has not yet been confirmed. We previously demonstrated, however, that long-term intake of 0.2% to 0.5% tea catechins had an antiobesity effect in mice (26), and the intra-abdominal fat area determined by computed tomography decreased following a 3-mo intake of 690 mg/day tea catechins in humans (30). In light of the findings of our previous antiobesity studies, a similar positive effect might be expected on aging-related changes in humans who drink sufficient quantities of catechins in combination with habitual exercise.

Perspective and Significance

Combining catechin intake with habitual exercise is beneficial for suppressing the aging-related decline in physical performance and energy metabolism, and these effects may be attributed, at least in part, to improved mitochondrial function in skeletal muscle. These results suggest that tea catechins

combined with habitual exercise might be useful for preventing a decline in physical function during human aging.

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