

Effects of Catechin-Ingestion and Heat Stress on the Maximum Isometric Force in Knee Extension, the Volume of Quadriceps Muscle, and Serum Thiobarbituric Acid Reactive Substances Level in Healthy Elderly Women

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Abstract

Background: Sarcopenia, is a major concern for our aged society because sarcopenia is a fundamental cause of frailty and functional disability.

Aim: The effects of catechin-ingestion and/or heat treatment on the volume in quadriceps muscles, isometric force of knee extension, and serum thiobarbituric acid reactive substances (TBARS) were investigated in healthy elderly women.

Methods: Subjects (71.0 ± 0.7 years old, n=27) were divided into 3 groups; 1) catechin-ingested, 2) heat-stressed, and 3) catechin-ingestion with heat stressed (catechin+heat) groups. Subjects in both catechin-ingested and catechin+heat groups daily ingested 350 ml of green tea containing 540 mg of catechin in one hour for 10 weeks. Heat stress was applied on the quadriceps muscles for 8 hours a day and 4 days a week by using two heat- and steam-generating sheets.

Results: In catechin+heat group, muscle volume (Pre: 670 ± 38.7 cm², Post: 676 ± 37.2 cm²) and maximal isometric force (Pre: 25.8 ± 2.5 kg, Post: 27.0 ± 2.5 kg) were significantly increased by the 10-week of treatment (p<0.05), but not in catechin-ingested and heat-stressed groups. The time of Timed-Get-Up-and-Go test (TUG), which is widely used to measure sit-to-stand performance, in both catechin-ingested (Pre: 6.70 ± 0.33 s and Post: 6.19 ± 0.28 s) and catechin+heat (Pre: 6.49 ± 0.30 s and Post: 5.95 ± 0.24 s) groups in catechin-ingested group; was significantly shortened by the treatment (p<0.05). Following 10-week of the treatment, serum TBARS levels in both catechin-ingested (Pre: 29.06 ± 1.32 nmol/ml, Post: 24.47 ± 2.30 nmol/ml) and catechin+heat (Pre: 29.84 ± 1.57 nmol/ml, Post: 23.92 ± 0.98 nmol/ml) groups were significantly decreased (p<0.05), but not in heat group.

Conclusions: Evidences suggest that catechin-ingestion with heat stress might improve impaired muscle function of elderly women.

Keywords: Skeletal muscle; Elderly women; Catechin; Heat stress; Thiobarbituric acid reactive substances

Introduction

Aging-related decline of muscle strength and mass, so-called sarcopenia, is a major concern for our aged society because sarcopenia is a fundamental cause of frailty and functional disability [1,2]. Several resistance exercise and/or nutritional interventions are offered as a countermeasure for inactivity-associated muscle atrophy and weakness [3,4]. However, an effective countermeasure for sarcopenia is not developed.

Reactive oxygen species (ROS) in skeletal muscle cells are suggested to induce an increase in proteolysis which leads to muscle atrophy [5,6]. In addition, ROS also depresses muscular strength [7,8]. Therefore, Oxidative stress is proposed as one of major causes of sarcopenia [9]. If oxidative stress in skeletal muscle is increased by aging, supplementation of antioxidant could prevent the age-related loss of muscle mass and strength.

Green tea, which is widely consumed in Asian countries, is rich in polyphenols such as epigallocatechin gallate (EGCG), epicatechin gallate (ECG), gallic catechin (GC), epigallocatechin (EGC). It has been reported that catechin has not only antioxidant activity but also stimulating action of lipid catabolism in experimental animals and humans [10-14]. It has been reported that catechin has anti-atrophy

effects on unloading-induced muscle atrophy [15]. Therefore, it is suggested that catechin ingestion could improve skeletal muscle function of elderly people if age-related muscle dysfunction is attributed to an increment of oxidative stress. However, the effects of catechin-intakes on the serum oxidant levels in elderly people remain unclear.

On the other hand, heat stress is one of hypertrophic stimuli for skeletal muscle [16,17]. It has been proposed that heat stress may be a countermeasure for prevention of muscle atrophy [18]. Recently, heat stress-associated skeletal muscle hypertrophy has been reported

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in aged (106-week old) mice [19]. These evidences strongly suggested that the application of heat stress could induce muscular hypertrophy and could improve muscle function in elderly people. However, there was no evidence showing that effects of heat stress on skeletal muscle in elderly people. In addition, the improvement of functional capacity of skeletal muscles in elderly people may be more enhanced by combined treatment of catechin-ingestion and heat stress, compared with the each treatment alone.

Although sarcopenia is a fundamental cause of frailty and functional disability in both men and women [20], sarcopenia in women develops earlier than in men [21]. In addition, it has been reported that sarcopenic elderly women were more likely to have low bone mineral density, muscle strength, and obesity [22]. It is well known that the number of patients having osteoarthritis of the knee by is larger in women than men. Sarcopenia is considered to be one of their causes. It has been also reported that oxidative stress is an independent predictor of decline in walking speed and progression to severe walking disability among older (65 years and older) women [23], and that increased oxidative stress is suggested to be contributing to loss of muscle strength in older (65 years and older) women [7].

In the present study, therefore, we investigated the effects of catechin-ingestion and/or heat stress on skeletal muscle mass and strength in elderly women. Responses of serum thiobarbituric acid reactive substances (TBARS), an oxidative stress marker, were also evaluated.

Materials and Methods

Subjects and grouping

Twenty seven healthy elderly women (Age: 67-79 years old, n=27) participated in the study (Table 1). All experimental procedures were conducted in accordance with World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects). The study was approved by the Bioethics Committee at Toyohashi SOZO University. All subjects were informed about the possible risks in this study, and a signed informed consent was obtained from each subject. None of the subjects was taking any drugs or food supplements. All subjects had no problem about their daily mobility, and were not participating in regular exercise program for at least 6 months prior to the start of the study. Further, the subjects did not also participate in any new training programs during the experimental period.

Subjects were randomly divided into 3 groups; 1) catechin-ingested (catechin), 2) heat-stressed (heat), and 3) catechin-ingestion with heat stressed (catechin+heat) groups so as to have the mean age of subjects in respective group equal. Body weights of all subjects did not change significantly during the experimental period (Table 1). In the present study, untreated control group was not set. Therefore, we compared the measurements following the effects of 10-week of catechin-ingestion with or without heating with those before the treatments.

Catechin-intake

Subjects in catechin-ingested and catechin+heat groups ingested 350 ml of Healthia Green Tea® containing 540 mg of green tea catechin in one hour every day for 10 weeks by themselves. Catechin, catechin gallate, GC, gallic acid, gallic acid gallate, epigallocatechin gallate, ECG, EGC, and EGCG, and caffeine contents in the test beverages were similar to those used in our previous study [14]. Subjects were also instructed to continue their usual food intake and exercise habits throughout the study period.

Application of heat stress

Subjects in heat-stressed and catechin+heat groups, two commercial heat- and steam-generating sheets (Kao Corporation, Tokyo, Japan) were placed on the thigh with the position with above knee laterally by using a commercial net bandage (supporter). Heated leg was chosen randomly for each subject. Dominant leg was heated in 5 out of 9 subjects in each group. The details on the heat- and steam-generating sheet are described elsewhere [24]. The heating was applied to the same area of leg for 8 hours a day and 4 days a week for 10 weeks by themselves.

Isometric force and muscle mass

Isometric force and muscle mass of thighs were measured bilaterally 1 day before the initiation of heat application and 3 day after the termination of 10-week treatment. The measurements were carried out by a well-trained staff before and after the treatment. The blood sampled from an antecubital vein before various measurements. All measurements were performed after an overnight fast.

Isometric force: Measurements of isometric force in both thighs were performed by using Force Measurement System for one Leg (T.K.K.5715, TAKEI Scientific Instruments, Co. Ltd., Nigata, Japan) equipped with tension meter D (T.K.K.5710e, TAKEI Scientific Instruments). Subjects performed a maximum voluntary extension at 90° of knee joint thrice isometrically. Each contraction was performed for 5 seconds with 5-second interval. In catechin-ingested group, the average of maximal force of both legs in each subject was determined. Maximal value among 3 trials was used for the maximal isometric force in this study.

Muscle mass: MRI was performed with a 3.0-Tesla MRI system (Trio, Siemens, Erlangen, Germany). T1 weighted morphological coronal images of both femurs were acquired to select axial slices for Dixon imaging. Three dimensional 2-point Dixon sequence was acquired in an axial plane, and in-phase, opposed phase, fat, and water image were obtained. The scanning parameters for Dixon sequence were shown in Table 2. In phase images (fat and water) were loaded

	Age (yrs)	Height (cm)	Body weight (kg)	
			Pre	Post
catechin	71.4 ± 1.3	153 ± 1.1	52.2 ± 2.9	51.5 ± 2.8
heat	71.0 ± 1.3	153 ± 1.5	52.3 ± 2.7	52.3 ± 2.7
catechin+heat	70.3 ± 1.0	155 ± 2.0	53.9 ± 3.1	54.0 ± 3.1

Values are means ± SEM.

n = 9 in each group.

catechin: catechin-ingested group

heat: heat-stressed group

catechin+heat: catechin-ingestion with heat stressed group

Pre: before the initiation of study

Post: 3 days after the last bout of heating

Table 1: Physical characteristics of subjects.

TR	12 msec
TE	2.5 and 3.7 msec
FOV	360 x 360 mm
Slice thickness	0.9 mm
Number of slices	320 slices
Matrix size	384 x 384
Excitation	1
Bandwidth	383 kHz
Total scan time for this sequence	about 8 min

Table 2: Scanning parameters for Dixon sequence.

into the commercially available software Dr. View (Asahikasei, Tokyo, Japan) to measure the volume of quadriceps muscles. For the percentage fat measurement and volume measurement, region of interest was drawn over the whole area of quadriceps muscles, and the average of fat percentage and sum of area, respectively, for all slices from 5 to 15 cm-proximal position of the upper edge of the patella were measured. The total number of slices used for the measurement was 111 slices.

Functional test

The Timed-Get-Up-and-Go test (TUG) was subjected to all participants to evaluate a muscular function [25]. TUG is widely used to measure sit-to-stand performance [26]. A well trained investigator measures the time between standing up to sitting down in seconds.

TBARS analyses

Blood was centrifuged at 1,050 g for 15 min, and then serum was collected. Serum TBARS was measured using a colorimetric kit (Oxi-Tek TBARS assay kit, Enzo Life Sciences, NY, USA).

Statistics and data analyses

All values were expressed as means \pm SEM. The normal distribution of the data was confirmed by using Kolmogorov-Smirnov test. Then, the statistical significance of the values was analyzed by 2-way repeated ANOVA (treatment: catechin ingestion and heat \times time) by using SPSS Statistics 17.0 (SPSS Japan, Tokyo, Japan) followed by Tukey-Kramer post-hoc test or paired t-test was performed. Statistical significance was established at $p < 0.05$.

Results

Muscle strength of knee extension

Maximal force of knee extension was shown in Figure 1a. In catechin+heat group, maximal force was significantly increased by the treatment ($p < 0.05$). However, there was no significant change in maximal force between before and after the treatment with not only Heat but also catechin-ingested groups.

Muscle volume

Following 10-week of the treatment, the muscle volume in catechin+heat group significantly increased ($p < 0.05$) in heated and unheated legs (Figure 1b). On the other hand, in both heat-stressed and catechin-ingested groups, no significant difference in the volume between before and after the 10-week-treatment was observed.

The time of TUG

Changes in the time of TUG were shown in Figure 2. In both catechin-ingested and catechin+heat groups, the time of TUG was significantly shortened by each treatment ($p < 0.05$). In heat-stressed group, there was no significant change in the time of TUG between before and after the treatment.

Serum TBARS

Following 10-week of the treatment, serum TBARS levels in catechin-ingested (\sim 15%) and catechin+heat (\sim 19%) groups significantly decreased ($p < 0.05$, Figure 3). Although the level of serum TBARS in heat-stressed group was also decreased by 10-week of treatment (\sim 7%), no significant difference was observed.

Discussion

In the present study, we investigated the effects of catechin-

ingestion and/or heat stress on the maximum isometric force in knee extension, the volume of quadriceps muscle, and serum TBARS level in elderly healthy women. This study showed a significant increase in the maximum force and the muscle volume with a significant decrease in the time of TUG and serum TBARS level were observed following 10-week of combined treatment of catechin-ingestion and heat stress compared with the values before the treatment. The present study also demonstrated that the decreases in the time of TUG and serum TBARS level were also induced by catechin-ingestion alone, but not by heat treatment. This is the first study showing that catechin-ingestion and/or heat stress could improve the skeletal muscle function of lower limbs and the level of oxidative stress in elderly healthy women. Skeletal muscle function in elderly healthy women might be improved by catechin-ingestion with heat stress, without physical exercise.

Effects of catechin-ingestion and heat stress on serum oxidative stress

In the present study, serum TBARS levels were significantly

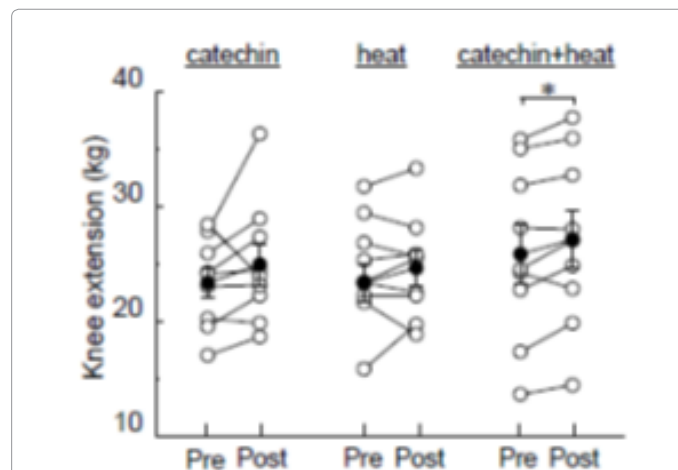
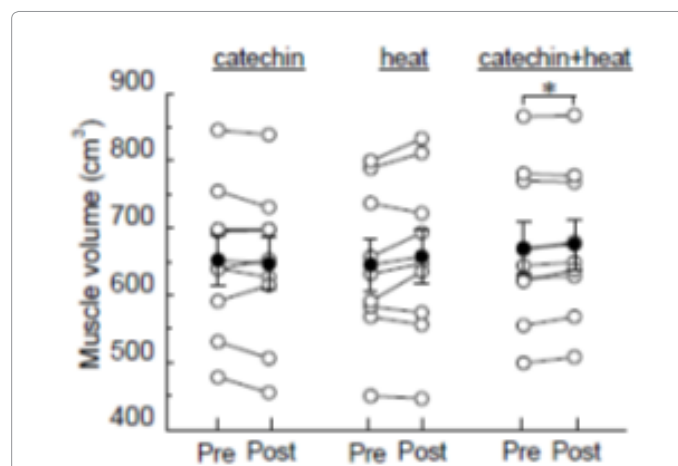


Figure 1a: Effects of catechin-ingestion and/or heat stress on maximal force of knee extension.



Opened circles with line show the changes in the maximal force and the muscle volume of each subject before and after 10-week treatment. Closed circles with line show the mean values with SEM before and after 10-week treatment. n=9 in each group. catechin: catechin-ingested group, heat: heat-stressed group, catechin+heat: catechin-ingestion with heat stressed group, Pre: before 10-week-treatment, Post: after 10-week-treatment. *: $p < 0.05$.

Figure 1b: Effects of catechin-ingestion and/or heat stress on muscle volume.

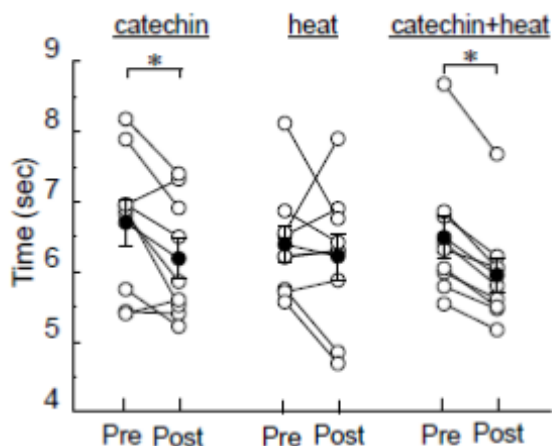


Figure 2: Effects of catechin-ingestion and/or heat stress on the time of the Timed-Get-Up-and-Go test (TUG). Opened circles with line show the changes in the time of TUG of each subject before and after 10-week treatment. Closed circles with line show the mean values with SEM before and after 10-week treatment. n=9 in each group. Abbreviations are the same as in Figure 1 *: p<0.05.

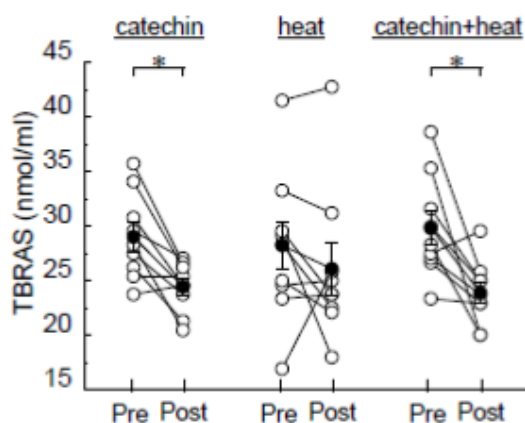


Figure 3: Effects of catechin-ingestion and/or heat stress on serum thiobarbituric acid reactive substances (TBARS) level. Opened circles with line show the changes in serum TBARS level of each subject before and after 10-week treatment. Closed circles with line show the mean values with SEM before and after 10-week treatment. n=9 in each group. Abbreviations are the same as in Figure 2. *: p<0.05.

decreased following 10-week of catechin-ingestion with or without the application of heat stress (-15 ~ -19%, p<0.05). However, heating alone had no significant effect on serum TBARS levels (~-7.7%). Therefore, daily catechin-ingestion depressed oxidative stress in healthy elderly women although the level of serum catechin was not evaluated. This result was consistent with the previous studies in type 2 diabetes patients [27] and in obese and hypertensive patients [28]. In healthy elderly women, however, this is the first report showing that the effects of catechin-ingestion and/or heat stress on serum TBARS level. Daily catechin-ingestion might have an antioxidant activity in elderly women.

Effects of catechin-ingestion on skeletal muscle mass and function

In the present study, catechin ingestion alone had no effects on

muscle volume of quadriceps muscles, and had small but insignificant effects on isometric force of knee extension. However, TUG, which is a physiological index of skeletal muscle function, was improved by catechin ingestion alone (p<0.05). Recently, catechin with exercise training was reported to have a beneficial effect on skeletal muscle function measured by TUG and skeletal muscle mass [29]. However, this is the first study showing that catechin-ingestion without exercise training could improve walking ability measured by TUG in healthy elderly women. It has also been reported that catechin attenuates the aging-associated loss of force production and oxidative stress in senescence-accelerated mice [30]. In addition, increased oxidative stress is suggested to be contributing to loss of muscle strength in older (65 years and older) women [7]. These observations suggest that aging-associated depression of muscle function in elderly women may be attributed to oxidative stress in skeletal muscle, in part.

Effects of heat stress on skeletal muscle mass and function

In the present study, small and insignificant increases of muscle volume in quadriceps muscles and isometric force of knee extension were observed following 10-week of heating. Previous study showed that 10-week of heat stress by using the same kind of heating sheet induces hypertrophy as well as increase of force generation of quadriceps muscle in healthy human subjects [16]. The discrepancy of the results from both studies may be due to the differences of heating area and subjects. Increment of muscle mass induced by heat stress is dependent on the muscle temperature [18]. Heating area (668 cm²) in the previous study was larger than that in the present study (96.04 cm²). Heating area may effect on muscle temperature during heating.

Another explanation is differences in age and sex of subjects. In the previous study, healthy middle-aged men participated (45.1 ± 2.0 years old). There is no report regarding the effects of heat stress on skeletal muscle in female animals and human subjects. Although we confirmed heat stress-associated muscle hypertrophy in aged mice recently [19], it is still unclear the effects of heat stress on skeletal muscle function in healthy elderly women.

Cumulative effects of catechin-ingestion and heat stress on muscle mass and function

In the present study, cumulative effects of the combined treatment of catechin-ingestion and heat stress on increments in muscle mass and strength of knee extension were observed in healthy elderly women. Local heating facilitates human skeletal muscle blood flow [31]. Application of heat stress might increase the delivery of the catechin-associated antioxidants in the heated quadriceps muscle, resulting that catechin-associated antioxidative effects may be enhanced by additional heat treatment. Further studies are needed to clarify the mechanism of the cumulative effect of heat treatment and green tea catechin intake on skeletal muscle.

Perspective and limitation

The present study showed that catechin-ingestion with heat stress improved muscle function with reduction of serum oxidative stress in elderly healthy women. It is still unclear whether these combined treatments could improve impaired skeletal muscle of elderly women with sarcopenia or not. If age-related muscle impairment is attributed to an increment of oxidative stress, catechin-ingestion with heating could be a useful countermeasure to improve skeletal muscle function of elderly people. However, additional studies need to elucidate the preventive effects of catechin-ingestion with heat treatment on sarcopenia.

Another limitation of this study is that the subjects were only women, and the findings cannot necessarily be extrapolated to men. In addition, it was also based on a small sample. Another problem is that this study was not a blind study. However, this is the first study to show the combined treatment of catechin-ingestion and heat stress application improves skeletal muscle function in elderly women.

Conclusion

The 10-week of catechin-ingestion with heat stress induced increases of the maximum isometric force in knee extension and the volume of quadriceps muscle of healthy elderly women. The combined treatment as well as catechin-ingestion alone also improved movement and depressed oxidative stress levels in serum. Evidences suggest that skeletal muscle function of elderly women might be impaired by a reduction of muscle mass and an increase of oxidative stress.

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Conflict of Interest

The authors have read the journal's policy and have the following conflicts: Drs. HO, NO, HK, MI, A Suzuki, A Shimotoyodome, and TH are employees of KAO Corporation, KG got research fund from KAO Corporation, who supported this study, in part. This does not alter the authors' adherence to all the Journal of Gerontology and Geriatric Research policies on sharing data and materials.

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