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ARTICLE

Green tea extract supplementation does not hamper endurance-training adaptation but improves antioxidant capacity in sedentary men

Yu-Chi Kuo, Jung-Charng Lin, Jeffrey R. Bernard, and Yi-Hung Liao

Abstract: The purpose of this study was to investigate the effect of green tea extract (GTE) supplementation combined with endurance training on endurance capacity and performance in sedentary men. Forty untrained men (age: 20 ± 1 years) participated in this study. Subjects were assigned to 1 of 4 treatments: (*i*) placebo-control (CTRL), (*ii*) GTE, (*iii*) endurance training (Ex), and (*iv*) endurance training with GTE (ExGTE). During the 4-week intervention, exercise training was prescribed as 75% oxygen uptake reserve for three 20-min sessions per week, and either GTE (250 mg/day) or placebo was provided. Endurance capacity, malondialdehyde (MDA), total antioxidant status (TAS), and creatine kinase (CK) were examined. Ex and ExGTE but not GTE improved exhaustive-run time (Ex: +8.2%, $p = 0.031$; ExGTE: +14.3%, $p < 0.001$); in addition, Ex and ExGTE significantly increased maximal oxygen uptake by ~14% ($p = 0.041$) and ~17% ($p = 0.017$) above the values of the CTRL group, respectively. Both Ex and ExGTE significantly decreased the increase of CK by ~11%–32% below that of CTRL following an exhaustive run (Ex: $p = 0.007$; ExGTE: $p = 0.001$). Moreover, TAS levels increased by ~11% in ExGTE after training ($p = 0.040$), and GTE, Ex, and ExGTE markedly attenuated exercise-induced MDA production ($p = 0.01$, $p = 0.005$, $p = 0.011$, respectively). In conclusion, this investigation demonstrated that daily ingestion of GTE during endurance training does not impair improvements in endurance capacity. Moreover, endurance training combined with GTE not only increases antioxidant capacity without attenuating endurance training adaptations, but also further attenuates acute exercise-induced CK release.

Key words: $\dot{V}O_{2max}$, malondialdehyde (MDA), creatine kinase (CK), oxidative stress, catechins.

Résumé : Cette étude se propose d'examiner l'effet de la supplémentation en extrait de thé vert (« GTE ») combinée à l'entraînement en endurance sur la capacité d'endurance et la performance chez des hommes sédentaires. Quarante hommes non entraînés (âge de 20 ± 1 ans) participent à cette étude. Les sujets sont assignés à l'un des quatre traitements : (*i*) placebo-contrôle (« CTRL »), (*ii*) GTE, (*iii*) entraînement en endurance (Ex) et (*iv*) entraînement en endurance plus GTE (ExGTE). Durant les quatre semaines de l'intervention, l'entraînement physique consiste en trois séances de 20 min à une intensité sollicitant 75 % du consommation d'oxygène de réserve dans la condition de GTE (250 mg/jour) ou de placebo. On évalue la capacité d'endurance, le taux de malonaldéhyde (« MDA »), le statut antioxydant total (« TAS ») et la créatine kinase (« CK »). Ex et ExGTE, mais pas GTE suscitent une amélioration du temps de course jusqu'à épuisement (Ex: +8,2 %, $p = 0{,}031$; ExGTE: +14,3 %, $p < 0{,}001$); de plus, Ex et ExGTE suscitent une augmentation significative du consommation maximale d'oxygène de ~14 % ($p = 0{,}041$) et de ~17 % ($p = 0{,}017$) respectivement par rapport au groupe CTRL. EX et ExGTE suscitent une baisse significative de l'augmentation de CK de ~11–32 % par rapport à CTRL à la suite de la course jusqu'à épuisement (Ex: $p = 0{,}007$; ExGTE: $p = 0{,}001$). De plus, TAS s'accroît de ~11 % dans la condition ExGTE suite à l'entraînement ($p = 0{,}040$); GTE, Ex et ExGTE atténuent nettement la production à l'effort de MDA ($p = 0{,}01$, $p = 0{,}005$, $p = 0{,}011$, respectivement). En conclusion, d'après cette étude, l'apport journalier de GTE durant l'entraînement en endurance ne nuit pas à l'amélioration de la capacité d'endurance. En outre, l'entraînement en endurance combiné à GTE n'améliore pas seulement la capacité antioxydante sans nuire aux adaptations dues à l'entraînement, mais atténue davantage la libération à l'effort de CK. [Traduit par la Rédaction]

Mots-clés : $\dot{V}O_{2max}$, malonaldéhyde (MDA), créatine kinase (CK), stress oxydatif, catéchines.

Introduction

Regular exercise training has been shown to improve physical fitness (Janssen and LeBlanc 2010) and to decrease the risks of chronic/degenerative diseases, such as obesity and diabetes (Mitchell and Barlow 2011; Booth et al. 2012). During strenuous and/or prolonged exercise, a significant amount of free radicals are produced by the working muscles, which can result in the rapid accumulation of systemic oxidative stress (Powers et al. 2011). The degree by which oxidative damage affects the human body are tightly regulated by the balance of free-radical production and their elimination by both endogenous and exogenous antioxidant defense systems (Haddock 1992). Therefore, it was previously believed that the use of exogenous antioxidants could favorably suppress oxidative damage and enhance training benefits (Sastre et al. 1992; Konig et al. 2001). However, the literature suggests that ingestion of traditional antioxidants (e.g., vitamins C and/or E) may impair the gains in maximal oxygen uptake ($\dot{V}O_{2max}$) and muscular adaptations following endurance training

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(Gomez-Cabrera et al. 2008; Strobel et al. 2011; Braakhuis et al. 2014; Close and Jackson 2014; Paulsen et al. 2014).

The negative impact of traditional antioxidants on exercise training adaptation raises an interesting question: would the use of natural antioxidants during exercise training have distinct effects? In this regard, exploring the scientific-based health benefits of natural extract during exercise training has thus become the focus of recent research into functional supplements. Emerging evidence indicates that green tea extracts (GTE) contain important polyphenol compounds (i.e., catechins) that underlie antioxidant mechanisms (Ruch et al. 1989; Yang et al. 2001; Higdon and Frei 2003), which could attenuate oxidative stress and enhance substrate metabolism. The catechins have been classified based on the number of hydroxyl groups within their chemical structures, including epicatechin (EC), gallocatechin, epigallocatechin (EGC), catechin gallate, gallocatechin gallate, epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) (Higdon and Frei 2003; Hodgson et al. 2013). Although many studies report positive effects of GTE on attenuating oxidative stress (Murase et al. 2008; Panza et al. 2008; Haramizu et al. 2011) and improving endurance capacity (Murase et al. 2005, 2006; Richards et al. 2010), not all studies agree (Eichenberger et al. 2009, 2010). Moreover, to date, there is still little known about whether endurance training combined with GTE ingestion would impair the gain in endurance capacity following training in a sedentary population.

Therefore, it is plausible that GTE supplementation during moderate endurance training can help sustain exercise-induced training adaptions and further improve antioxidant capacity. For this investigation we employed a double-blind plus placebo/sedentary control design to investigate the effects of 4-week endurance training combined with GTE supplementation on endurance capacity in sedentary men. Furthermore, we also examined total antioxidant capacity and muscle damage following an acute exercise challenge.

Methods and materials

Subjects

Forty healthy male individuals volunteered to participate in this study. The subjects averaged 20 ± 1 years of age, 66.0 ± 8.1 kg in body mass, and 172.1 ± 5.5 cm in height. The subjects had no previous regular exercise training and had not participated in organized sports for at least 3 months prior to this study. Following an explanation of the study purpose, experimental procedures, supplements to be used, the potential risks involved, and completing a health screening questionnaire, study participants provided both verbal and written informed consent. In accordance with the health screening questionnaire, all subjects were free of dyslipidaemia, diabetes mellitus (types 1 and 2), hypertension, cardiovascular diseases, and/or other metabolic disorders. Study procedures were approved by the Human Subject Ethics Committee of National Taiwan Normal University (Taipei City, Taiwan) before the subject recruitment. To ensure the quality of the study all researchers were blinded to the respective treatments; however, they were still responsible for reminding each participant to take their supplements.

Experimental design and testing procedures

A double-blind and placebo-controlled experimental design was used to ensure the integrity of the study. The experimental procedures consisted of 3 primary sections: (*i*) pre- training screening, (*ii*) 4-week endurance training with or without catechins supplements, and (*iii*) the exercise-testing day after the completion of endurance training. All participants, including placebo-control (CTRL) group, performed a familiarized exhaustive-run exercise at least 1 week before pre- and post-training test to eliminate learning effects. During the pre-training test section, the initial $\dot{V}O_{2max}$ and run-time to exhaustion of all the subjects were determined (both tests were separated by 2 days) using a modified treadmill-exercise protocol (Froelicher et al. 1974; Brooks et al. 1996). Thereafter, subjects were assigned to 1 of 4 experimental groups by matching their pre-training $\dot{V}O_{2max}$ values: (*i*) CTRL (n = 10), (*ii*) GTE-supplemented group (n = 10), (*iii*) endurance-training group with placebo (Ex; n = 10), and (*iv*) endurance training with GTE supplement group (ExGTE; n = 10) (Table 1).

Following the pre-training tests, subjects next underwent a 4-week endurance exercise training program combined with either GTE supplement (250 mg GTE/(capsule·day) with breakfast in the morning (Numen Biotechnology Co. Ltd, Taipei, Taiwan) or placebo (starch capsule, equivalent amount to catechin; Taiwan Sugar Corp., Tainan, Taiwan). During the 4-week intervention, the compliances to GTE ingestion and endurance training were evaluated by a general health screening questionnaire provided by the researchers. At least 24 h after the end of the 4-week endurance training program (intensity: 75% oxygen uptake reserve ($\dot{V}O_{2R}$); duration: each for 20 min; frequency: 3 times/week), a maximal exercise test was performed to determine subjects post-training $\dot{V}O_{2max}$. Two days after the $\dot{V}O_{2max}$ test, fasting blood samples were collected and the run-to-exhaustion exercise challenge was performed by all subjects. Thereafter, postexercise blood samples were drawn immediately after exercise, and in addition, postexercise recovery blood samples were drawn 24 h after the maximal exercise challenge test. Blood samples were then centrifuged at 1500 RCF for 10 min (4 °C), the plasma collected and stored at −80 °C for the analyses of malondialdehyde (MDA), total antioxidant status (TAS), and creatine kinase (CK) activity.

GTE and composition of active catechins

For the GTE supplement, the composition of individual catechin component was analyzed using high-performance liquid chromatography/electrospray ionization mass spectrometry method (HPLC-ESI-MS). GTE sample was obtained from Numen Biotechnology (Numen Biotechnology Co. Ltd), and the standards for GTE active components (catechins: EGC, EGCG, EC, and ECG) were obtained from Sigma–Aldrich (Sigma–Aldrich Inc., St. Louis, Mo., USA). The compositions of EGC, EGCG, EC, and ECG, in the GTE were 14.7%, 48.2%, 6.9%, and 13%, respectively (total tea catechins = 82.8%).

$\dot{V}O_{2max}$ test and running time to exhaustion

$\dot{V}O_{2max}$ was performed on a motor-drive Quinton 65 treadmill (Quinton Instrument, Seattle, Wash., USA), and real-time breath-by-breath gas samples were analyzed using a Vmax-29 metabolic measurement cart (SensorMedics, Yorba Linda, Calif., USA). For the $\dot{V}O_{2max}$ test the following criteria were used to determine that a participant had achieved his $\dot{V}O_{2max}$: (*i*) respiratory exchange ratio greater 1.2, (*ii*) heart rate (HR) reaching predicted maximal HR (220 − age), and (*iii*) the plateau of oxygen consumption with increasing working load. For evaluating run-time to exhaustion, a modified treadmill exercise protocol (Froelicher et al. 1974; Brooks et al. 1996) was used and performed on a motor-driven treadmill (Quinton Instrument). Maximal exhaustion during incremental run exercise was identified by the point at which the subject was unable to maintain the required pace on the treadmill. As a secondary measurement, we also assessed their HRs during exercise to ensure they reached their maximal efforts. The terminal speed and incline of exhaustive run for all participants were ranged from 8.1–9.7 km/h and 18%–20% grade (stage 5–7).

Analyses of biochemical biomarkers for oxidative stress and muscle damage

Plasma levels of TAS and CK were analyzed using commercially available methods (TAS: Randox NX2332 kit; Randox, Crumlin, UK; CK: Roche Products, UK) on a Cobas Mira Plus analyzer (Roche Diagnostic Systems) and an automated system (Cobas Mira Plus; Roche Diagnostic Systems), respectively (Teixeira et al. 2009). The

Table 1. Basic characteristics of subjects at baseline.

Treatment (group)	Age (y)	Height (cm)	Body mass (kg)
CTRL	20±1	172.8±4.5	69.5±9.9
GTE	20±1	171.0±5.6	64.1±7.5
Ex	20±1	172.5±6.0	63.3±6.7
ExGTE	21±1	172.0±6.0	67.2±8.2

Note: CTRL, placebo-control group (n = 10); GTE, green tea extract supplement group (n = 10); Ex, endurance exercise training group with placebo (n = 10); ExGTE, endurance training with green tea extract supplement group (n = 10).

coefficient of variation (CV%) for TAS intra-assay and inter-assay were 3.4% and 3%, respectively; in addition, the CV% for CK intra-assay and inter-assay were 3.5% and 3.1%, respectively. Plasma MDA was analyzed using a photometer (GENSYS10 series Spectro Photometer analyzer, No.437634, Calbiochem, Calbiochem & Novabiochem Corp., La Jolla, Calif., USA). Briefly, MDA was measured in accordance with lipid peroxidation, which was quantified by determining the formation of thiobarbitturic acid reactive substances as previously described (Esterbauer and Cheeseman 1990). The CV% for MDA intra-assay and inter-assay were 5.5% and 5.9%, respectively.

Statistical analysis

All data sets were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, Ill., USA). In addition, subjects' normality were determined using the Kolmogorov–Smirnov test. A 1-way ANOVA with repeated-measures was used to compare the mean differences among all measured values of basic characteristics of subjects at baseline level. The percentage change between pre- and post-training in all measured values and parameters were calculated as [(post value − pre value)/pre value] × 100%. The differences of percentage changes among groups were analyzed using a 2-way ANOVA (GTE factors × endurance training factor), and a Fisher's multiple comparisons test was applied for post hoc analyses to compare the significant differences among groups. Pearson correlation analyses were used to evaluate the relationships among the improvement in $\dot{V}O_{2max}$ after the 4-week exercise training program and biomarkers for muscle damage (CK), systemic oxidative stress (MDA), or TAS. Statistical significance levels were set at $p < 0.05$. All values were expressed as the mean ± SE.

Results

Basic characteristics of subjects

All 40 subjects, 10 subjects in each experimental group, were able to complete the entire study. In addition, there were no statistical differences on variables of age, height, or body weight among treatment groups before the experimental intervention was performed (Table 1). During the 4-week treatment, no adverse effects on health (e.g., gastrointestinal discomfort, diarrhoea, etc.) were reported when GTE was administered alone or combined with exercise training.

Effects of exercise training and catechins on endurance exercise capacity

The absolute values of exhaustive-run time and $\dot{V}O_{2max}$ at pre- and post-intervention are shown in Table 2. There were no differences in $\dot{V}O_{2max}$ and run-time to exhaustion among groups prior to the intervention. After the 4-week intervention, in comparison with the CTRL group, a markedly longer exhaustive-run time was achieved by the Ex (p = 0.019) and ExGTE groups ($p < 0.001$). The ExGTE group also showed significant longer run-time to exhaustion compared with those in the GTE group (p = 0.001). The percent changes between pre- and post-intervention (Δ% between pre- and post-training) run-time to exhaustion are shown in Fig. 1A. There

Table 2. Endurance exercise performance at pre- and postexperimental intervention.

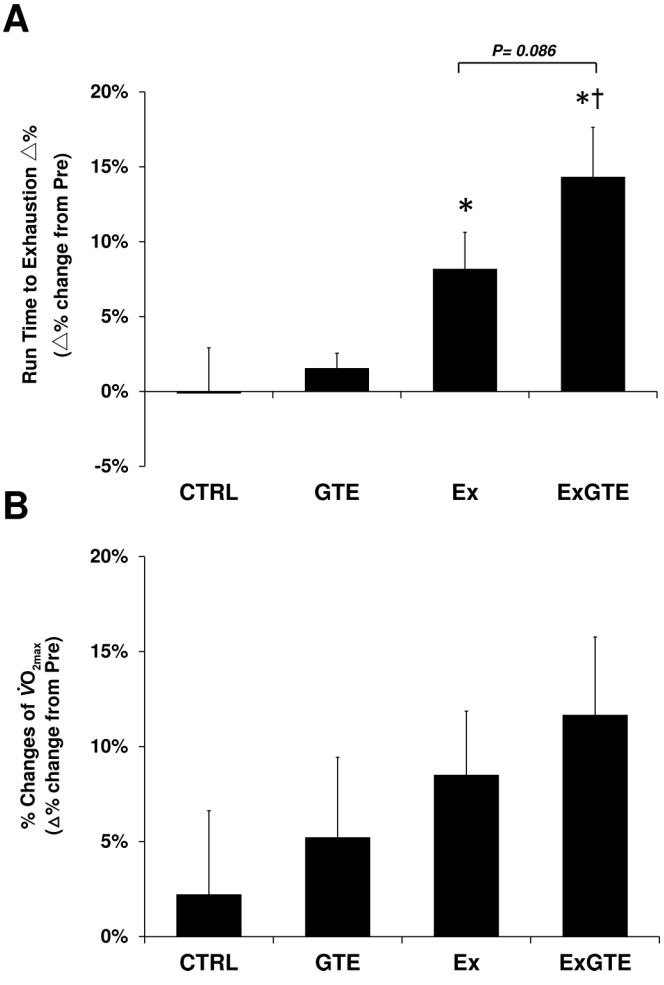
	$\dot{V}O_{2max}$ (mL/(kg·min))		Run time to exhaustion (s)	
Treatment	Pre	Post	Pre	Post
CTRL	41.3±1.5	41.9±1.5	936.9±13.0	934.0±24.8
GTE	41.4±1.0	43.6±2.2	936.7±17.9	950.8±18.8
Ex	44.2±1.9	48.0±2.5*	939.0±10.1	1014.2±17.4*
ExGTE	44.1±1.2	49.1±1.8*	937.0±13.2	1069.9±29.6*,†

Note: See Table 1 legend for treatment abbreviations. $\dot{V}O_{2max}$, maximal oxygen uptake.

*Significant differences in compared with the CTRL group ($p < 0.05$).

†Significant differences in compared with the GTE-supplemented group (catechine group; $p < 0.05$).

Fig. 1. Percent changes in measurements of endurance exercise capacity after 4-week intervention. Percent changes in run time to exhaustion (A) and percent changes in maximal oxygen consumption ($\dot{V}O_{2max}$) (B) measured by treatments at pre- (baseline) and postexperimental intervention (4-weeks of exercise training/green tea extract (GTE) ingestion) at pre- and postexperimental intervention (B). CTRL: placebo-control group (n = 10); GTE: GTE-supplemented group (n = 10); Ex: endurance exercise training group with placebo (n = 10); ExGTE: endurance training with GTE supplement group (n = 10). Values are means ± SE *, Significant differences in compared with the CTRL group ($p < 0.05$); †, significant differences in compared with the GTE group ($p < 0.05$).



were no interactions for Ex and GTE factors in exhaustive-run time, and the Ex and ExGTE groups showed significantly improved run-time to exhaustion compared with the CTRL group (Ex: +8.2%, $p = 0.031$; ExGTE: +14.3%, $p < 0.001$). Furthermore, the difference between the Ex and ExGTE groups approached statistical significance ($p = 0.086$). However, the group taking GTE alone did not show a difference compared with the CTRL group.

After the 4-week intervention, there were no interactions for Ex and GTE factors in $\dot{V}O_{2max}$, while both Ex and ExGTE groups showed significant increases in $\dot{V}O_{2max}$ by ~14% ($p = 0.041$) and ~17% ($p = 0.017$) above the values of the CTRL group, respectively (Table 2). Although the percent changes in $\dot{V}O_{2max}$ before and after training were not different among experimental groups, it did exhibit a pattern that would suggest exercise training combined with GTE may improve $\dot{V}O_{2max}$ (Fig. 1B).

Effects of exercise training and GTE on systemic oxidative stress

The results of plasma CK, MDA, and TAS before and after training are shown in Table 3. At the end of intervention, fasting plasma CK, MDA, and TAS levels were unaffected among groups. However, the percent changes in TAS levels (Δ% between pre- and post-training) significantly increased by ~11% above the levels of the CTRL in ExGTE groups ($p = 0.040$).

Biomarker of muscle damage in response to acute exhaustive-run exercise

For determining muscle damage status in response to exercise, plasma CK levels were measured before and 24 h after exhaustive-run exercise. The results are shown in Fig. 2A. The percent changes in circulatory CK levels increased by ~81% above baseline in the CTRL group at 24 h after exhaustive-run exercise, indicating a great increase in muscle damage resulting from exercise challenge. The degree of increase in plasma CK levels were only +40%, +26%, and +10% in GTE ($p = 0.038$), Ex ($p = 0.007$), and ExGTE ($p = 0.001$), respectively.

Systemic oxidative stress in response to exhaustive exercise

The results of percent changes in plasma MDA levels before and after an exhaustive-run exercise are shown in Fig. 3A. Twenty-four hours after exhaustive-run exercise, plasma MDA was increased by ~24% above pre-exercise level in the CTRL group. However, GTE, Ex, and ExGTE markedly attenuated their exercise-induced MDA response by ~90%–100% compared with the CTRL group (GTE: $p = 0.01$; Ex: $p = 0.005$; ExGTE: $p = 0.011$), but no additive effects were observed. The results of the percent change in plasma TAS levels before and after the exhaustive-run exercise are shown in Fig. 3B. Although there was a pattern to suggest that TAS decreased in response to exhaustive-run exercise, no differences were observed among treatment groups.

Correlation analyses

Here we determined whether there were any relationships among the improvement in $\dot{V}O_{2max}$ after 4-week training, biomarkers for muscle damage (CK), systemic oxidative stress (MDA), and/or TAS. There was a significant negative relationship between percent changes in $\dot{V}O_{2max}$ after training and percent change in plasma CK levels in response to exhaustive exercise ($r = -0.275$; $p = 0.043$) (see Fig. 2B). Furthermore, a significant negative relationship was observed between percent changes in plasma TAS and percent change in plasma CK levels in response to exhaustive exercise ($r = -0.319$; $p = 0.022$). However, no significant correlations were observed between percent change in $\dot{V}O_{2max}$ after training and percent change in plasma MDA ($r = 0.062$; $p = 0.352$) or TAS ($r = -0.013$; $p = 0.467$) levels in response to exhaustive exercise.

Table 3. Circulating levels of muscle damage- and oxidative stress-related biomarkers at pre- and postexperimental intervention.

Biomarkers	Pre	Post	Δ% Pre–Post
Plasma CK (IU/L)			
CTRL	132.4±7.9	145.0±13.3	10.0±7.7
GTE	121.2±12.0	111.1±12.2	−6.9±6.6
Ex	134.6±16.0	121.7±15.5	−7.4±6.6
ExGTE	123.6±19.0	121.9±14.9	2.9±7.3
Plasma MDA (μmol/L)			
CTRL	1.25±0.02	1.24±0.03	−1.3±2.6
GTE	1.27±0.02	1.25±0.03	−1.4±3.1
Ex	1.26±0.02	1.27±0.02	0.5±1.6
ExGTE	1.28±0.03	1.22±0.02	−3.9±1.9
Plasma TAS (mmol/L)			
CTRL	1.32±0.04	1.30±0.02	−0.7±3.8
GTE	1.29±0.05	1.32±0.04	4.2±5.7
Ex	1.26±0.02	1.29±0.03	2.5±2.7
ExGTE	1.27±0.02	1.39±0.04	9.9±3.0*

Note: See Table 1 legend for treatment abbreviations. CK, creatine kinase; D% Pre-Post: percent changes of values between pre- and postexperimental intervention; MDA, malondialdehyde; TAS, total antioxidant status.

*Significant differences in compared with the CTRL group ($p < 0.05$).

Discussion

The primary findings of the present investigation was that provision of GTE supplement (250 mg/day; equivalent to ~207 mg catechins) during a 4-week moderate-endurance training (intensity: 75% $\dot{V}O_{2R}$; duration: 20 min; frequency: 3 times/week) did not impair the gains of endurance capacity following training. Moreover, GTE supplement combined with endurance training had a clear protective effect on acute exercise-induced muscle damage and oxidative stress in sedentary young men. These results suggest that endurance training combined with GTE not only increases total antioxidant status without attenuating the endurance training adaptation, but also further improves antioxidant capacity to cope with an acute exercise challenge.

More recent evidence reveals that ingestion of traditional antioxidants (e.g., vitamins C and/or E) attenuates the gains in exercise training adaptations (Gomez-Cabrera et al. 2008; Strobel et al. 2011; Braakhuis et al. 2014; Close and Jackson 2014; Paulsen et al. 2014). Gomez-Cabrera et al. (2008) reported that daily vitamin C supplementation (1000 mg/day) suppressed improvements in endurance capacity after exercise training, suggesting that supplementation with vitamin C lowers training efficiency. Likewise, a more recent publication by Paulsen and colleagues (2014) also showed that provision of a vitamin C/E supplement (vitamin C: 1000 mg/day; vitamin E: 235 mg/day) markedly attenuated increases in markers of mitochondrial biogenesis following endurance training in recreationally endurance-trained individuals. These studies suggest that daily supplementation of antioxidants during exercise training might blunt reactive oxygen species-mediated mitochondrial biogenesis and subsequent gains in endurance capacity.

Interestingly, Braakhuis et al. (2014) reported that daily ingestion of blackcurrant juice (15 mg vitamin C/300 mg anthocyanins) during high-intensity training improved exercise performance in trained female runners, whereas vitamin C (1000 mg/day) decreased training benefits in this population. These findings raise the possibility that a natural antioxidant, supplemented at a lower dose, may not generate factors that would negatively impact the prospective gains in exercise induced adaptations following training. However, there is little attention given to whether GTE would impair adaptive responses to endurance training in sedentary population. In this study, we demonstrated that the gains in endurance-training adaptations (i.e., $\dot{V}O_{2max}$ and endurance performance) were sustained when GTE was ingested daily.

Fig. 2. Percent changes in circulating levels of creatine kinase in response to an exhaustive-run exercise after 4-week intervention. Percent changes in circulating level of creatine kinase (CK) measured by treatments before and 24 h after an acute bout of exhaustive-run exercise (A) and correlation between percent changes in circulating levels of CK before and 24 h after exhaustive-run exercise and percent changes in $\dot{V}O_{2max}$ after endurance training in 40 participants (Pearson = −0.275; $P = 0.043$) (B). See Fig. 1 legend for treatment abbreviations, and values are means ± SE. *, Significant differences compared with the control group (CTRL group; $p < 0.05$). b/t, between; PostEx, postexercise; PreEx, pre-exercise.

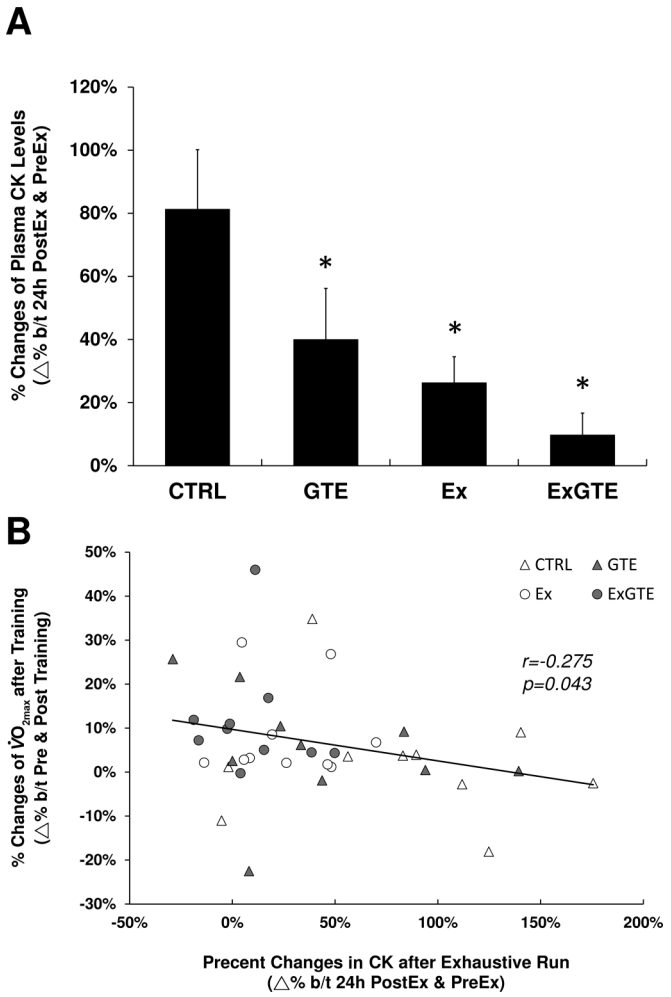
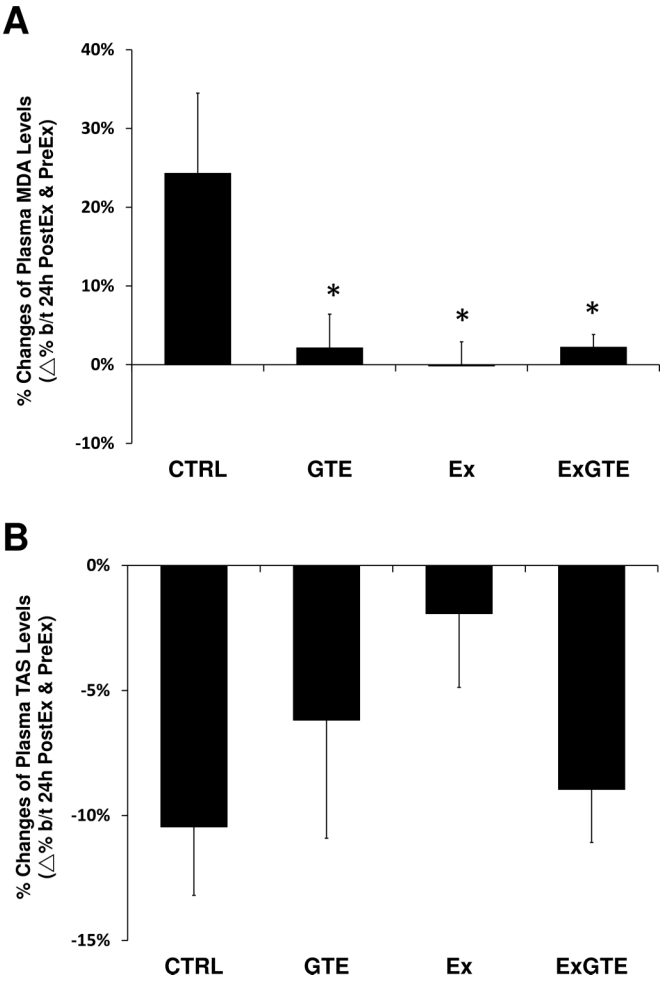


Fig. 3. Percent changes in circulating oxidative stress and total antioxidant status levels in response to an exhaustive-run exercise after 4-week intervention. Percent changes in circulating level of malondialdehyde (MDA) (A) and percent changes in circulating level of total antioxidant status (TAS) (B) measured by treatments before and 24 h after an exhaustive-run exercise. See Fig. 1 legend for treatment abbreviations, and values are means ± SE. *, Significant differences compared with the control group (CTRL group; $p < 0.05$). b/t, between; PostEx, postexercise; PreEx, pre-exercise.



Endurance training combined with GTE supplementation demonstrated a trend that would suggest greater improvement in endurance performance compared with endurance training alone (Ex: +8.2% vs. ExGTE: +14.3%; $p = 0.086$). When supplementing with GTE, there is the possibility that it may promote oxygen consumption and fat utilization in skeletal muscle. Richards et al. reported that short-term consumption of EGCG (473 mg/day for 2 days) increased $\dot{V}O_{2max}$ without affecting maximal cardiac output, suggesting that EGCG may promote muscle oxygen consumption during exercise (Richards et al. 2010). Furthermore, Murase and colleagues (2005, 2006) demonstrated that exercise training (5 days/week) plus GTE feeding significantly prolonged endurance exercise time by ~8%–24% and that this improvement might be mediated through increased lipid oxidation in rodent skeletal muscle (Murase et al. 2005, 2006). Likewise, Ichinose et al. (2011) also reported that daily GTE ingestion (572.8 mg/day), in combination with endurance training, was beneficial to promote fat utilization during exercise in human. The ability to enhance availability and utilization of free fatty acids during exercise would be crucial in sparing intramuscular glycogen and to reduce lactate production, which would positively contribute to an improvement in endurance capacity (Holloszy and Coyle 1984). Although we did not measure fat oxidation during exhaustive-run exercise in this study, several studies have reported the benefits of catechins on promoting lipid oxidation and decreasing diet-induced obesity in rodents (Murase et al. 2002, 2005, 2006; Shimotoyodome et al. 2005). Collectively, these results suggest that the improved endurance capacity observed in the present investigation may be, at least in part, mediated through enhanced fat oxidation in skeletal muscle.

However, not all studies have shown consistent results (Eichenberger et al. 2009, 2010). For example, previous human studies showed that 3-week GTE treatment (160 mg catechins/day equivalent to ~70 mg EGCG/day) did not alter indices of fat metabolism in endurance-trained men (Eichenberger et al. 2009, 2010). Moreover, 7 days of GTE ingestion (1136 mg catechins/day equivalent to ~624 mg EGCG/day) markedly increased lipolysis during prolonged moderate exercise (60-min cycle, 50% maximal

aerobic power), whereas fat oxidation was not affected (Randell et al. 2013). Taken together, the discrepancies observed in improved endurance capacity in response to exercise training and GTE ingestion may be due to (*i*) the use of animals or humans, (*ii*) trained or untrained subjects, (*iii*) training durations and protocols, (*iv*) supplement type (e.g., GTE vs. purified catechins), (*v*) administration dosage, (*vi*) the choice of markers of oxidative stress, and (*vii*) the use of muscle versus blood markers.

To date, the underlying mechanisms for the benefits of GTE or catechins combined with exercise training on endurance capacity are not fully understood. One possibility could be due to the effect of catechins on promoting mitochondrial biogenesis and fatty acid β-oxidation in muscle tissue (Holloszy and Booth 1976; Murase et al. 2008). Yet another possibility is that the ingestion of catechins may enhance endurance capacity via activation of pathways controlling mitochondrial biogenesis (Baar et al. 2002; Lee et al. 2006; Murase et al. 2008, 2009). These effects of catechins on promoting mitochondrial biogenesis may help to explain why the attenuated training efficiency in response to chronic vitamin C/E administration was not seen in studies employing catechins- or GTE-based supplements. However, more measurements (e.g., muscle biopsy, expression of mitochondrial enzymes, or fat oxidation during exercise) would be required to elucidate the precise underlying mechanisms for the benefit of catechins in humans.

Exercise training per se has been recognized as an antioxidant against oxidative stress; moreover, exercise-induced endogenous antioxidant capacity exerts beneficial effects in the prevention of chronic disease processes (Warburton et al. 2006). Of interest, we observed that the percent change in plasma TAS levels after 4-week intervention significantly increased by ~11% in ExGTE but not in either the Ex or GTE treatments. Our finding suggests that endurance training when combined with GTE supplementation further improves total antioxidant capacity. This is in agreement with a previous study that showed 7-day GTE ingestion alone, without exercise training, did not alter total antioxidant activity (Kimura et al. 2002). Because circulating TAS has been reported to be positively associated with $\dot{V}O_{2max}$ (Kostka et al. 2000), it is possible that the enhanced TAS might, at least in part, account for the improvements in endurance capacity. Furthermore, we herein demonstrated that a daily GTE supplementation was capable of increasing total antioxidant status without attenuating the endurance training adaptation in sedentary men, showing the potential benefits of providing this natural-sourced supplement during endurance training. Therefore, this raises the possibility that the combination of GTE and endurance training might be advantageous for maximizing exercise training adaptive benefits.

The protective effect of GTE ingestion during exercise training on muscle tissue might account for maintaining the gain in endurance capacity (Haramizu et al. 2011, 2013). We found that the release of CK was suppressed by the combined treatment of endurance training and GTE supplementation in an additive manner; moreover, there was a negative relationship between $\dot{V}O_{2max}$ improvement and increment of CK following exhaustive run. Because exercise-induce lipid peroxidation could increase membrane permeability and efflux of CK into the plasma (Kanter et al. 1988; Maughan et al. 1989), our results suggest that endurance training combined with catechins might better maintain membrane integrity and thereby increased oxygen consumption in muscle tissue.

However, it must be noted that no additive effects for additional attenuation of MDA production following the exercise challenge was observed in the ExGTE group. Perhaps collecting the blood when there was a significant difference in plasma MDA levels, presumably immediately after acute exercise challenge, would have revealed a positive additive effect of endurance training combined with GTE supplementation.

Conclusion

In summary, we observed that daily ingestion of GTE during endurance training does not impair the gains of endurance performance and $\dot{V}O_{2max}$ following training in sedentary men. Our results also demonstrate that a daily GTE supplementation not only increases antioxidant capacity without attenuating the endurance training adaptation, but also further attenuates acute exercise-induced CK release. Although endurance training and GTE both suppress acute exercise-induced MDA production, no additive effects were observed. However, future studies are warranted to investigate the precise mechanisms for the benefits of GTE supplementation in humans.

Conflict of interest statement

The authors declare no conflict of interests.

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