RESEARCH ARTICLE

Dietary apple polyphenols have preventive effects against lengthening contraction-induced muscle injuries

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We examined whether polyphenols from dietary apple have protective effects against exercise-induced muscle strain injury. Sixteen male Wistar rats were randomly assigned into the apple polyphenol (APP; $N = 8$) and control (CON; $N = 8$) groups. The APP and the CON groups were fed diets with and without 5% APP, respectively. After a 3-wk feeding period, the gastrocnemii of the animals were subject to lengthening contractions with electrical stimulation and forced ankle dorsiflexion. Isometric torques were measured before and after the lengthening contractions and on days 1, 2, 3, 5, and 7 after the contractions. On day 7, the animals were sacrificed and the gastrocnemii harvested. Thiobarbituric-acid-reactive substances, protein carbonyl, and mRNA of antioxidative proteins in the muscles were quantified. The APP group had significantly lower torque deficits than the CON group on days 3, 5, and 7 after the eccentric contractions. The thiobarbituric-acid-reactive substances and protein carbonyl levels in the case of the APP group were significantly lower than those in the case of the CON group. The APP group had significantly higher glutathione-*S*-transferase α1 mRNA levels than the CON group. Therefore, we conclude that dietary APPs have protective effects against lengthening contraction-induced muscle injury.

Keywords:
Apple / Injury / Lengthening contraction / Polyphenol / Skeletal muscle

1 Introduction

Apples account for a major percentage of the world's fruit supply. Procyanidin (proanthocyanidin) is the major polyphenol in apple [1, 2]. Procyanidin consists of (+)-catechin and (−)-epicatechin units and occurs widely as a secondary metabolite in plants. Polyphenols have a C6-C3-C6 backbone structure and are ubiquitous in the plant kingdom. Apple polyphenols (APPs) and purified procyanidin have various activities, such as antioxidant activity [3–5], modulating immune function [1, 6], and promoting adipose-tissue loss [7–9]. In addition to these biological functions, we showed that dietary APPs have beneficial effects on skeletal muscle function, such as elevating isometric tetanic torque in the ankle joint and ameliorate exercise-induced fatigue in the rat gastrocnemius [10].

The relationship between dietary polyphenols and skeletal muscle disease has also been reported. Buetler *et al.* and Dorchies *et al.* showed that green tea extract, which is rich in tea polyphenols, decreased muscle necrosis and elevated twitch tension in mdx dystrophic mice [11, 12]. Degenerative processes in dystrophic muscles are usually accompanied or even caused by alterations in calcium homeostasis, redox imbalance, and inflammatory processes. With regard to

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Abbreviations: APP, apple polyphenol; **CAT,** catalase; **Ct,** critical threshold; **GSTα1,** glutathione-S-transferase α1; **MAPK,** mitogen-activated protein kinase; **PC,** protein carbonyls; **RT-PCR,** real-time PCR; **SOD,** superoxide dismutase; **TBARS,** thiobarbituric-acid-reactive substances

polyphenols, their antioxidative properties are responsible for the beneficial effects against dystrophic atrophy, especially in terms of maintaining the redox balance.

Oxidative stress also induces and/or exacerbates exercise-induced muscle injury, such as strain injury [13, 14]. Aoi *et al.* showed that muscle damage in mice after prolonged treadmill exercise was associated with a redox-sensitive inflammatory cascade that was regulated by oxidative stress [15]. Uchiyama *et al.* also showed that weight-lifting exercise in rat increased superoxide dismutase (SOD), glutathione-*S*-peroxidase, and catalase (CAT) activities in the muscle [16]. Elevation in the levels of oxidative stress biomarkers (protein carbonyls (PC) in the plasma, plasma malondialdehyde, and 8-hydroxy-2′-deoxyguanosine) has also been confirmed after exercise in humans [13, 17, 18] and animals [19, 20].

On the other hand, it has been reported that exercise-induced oxidative stress is partially ameliorated by functional foods [21]. Aoi *et al.* showed that the antioxidant astaxanthin and a fermented milk diet reduces the levels of muscle-damage markers, such as serum creatine phosphokinase, thiobarbituric-acid-reactive substances (TBARS), and cytokine-induced neutrophil chemoattractant-1, induced by prolonged-treadmill exercise in mice [22, 23]. They also confirmed that the levels of protective proteins, such as antioxidant proteins (SOD2, glutathione-*S*-transferase α1 (GSTα1), and CAT), were higher in the case of animals fed with the test foods. Kato *et al.* also showed that the supplementation of the diet with antioxidant flavonoids suppressed treadmill exercise-induced oxidative stress in the rat skeletal muscle by using monoclonal antibodies against the oxidant of *N*-(hexamomyl) lysine [24]. Supplementation of the diet with vitamin C and E also attenuated the exercise-induced rise in the level of biomarkers (PC, malondialdehyde, and 8-hydroxy-2′-deoxyguanosine) of oxidative stress in humans [25–28]. These reports led us to consider that dietary APPs also have protective effects against oxidative stress, and thus, prevent exercise-induced muscle injuries.

In this report, we raised the hypothesis that dietary APPs have protective effects against exercise-induced muscle strain injury. We have previously reported a lengthening contraction-induced strain injury model: the rat gastrocnemius [29]. We measured the isometric tetanic torque, which is regarded as the most prominent measure of muscle strain injury [29]. TBARS and PC levels in the gastrocnemius were also examined to estimate oxidative stress as reported previously [30]. We further evaluated the mRNA expression of SOD1, SOD2, SOD3, GSTα1, and CAT to evaluate their role in the maintenance of the redox balance.

2 Materials and methods

All the procedures used in this study were approved by the ethical committee of the Nippon Sport Science University (permission number: 008-A01). All the experiments were performed by two independent operators.

2.1 APPs

APPs were extracted from unripe apples (*Malus pumila* cv. Fuji). APPs used in this study were a mixture of polyphenols that consist mainly of dimers to pentadecamers of procyanidin (approximately 45% w/w). The other components of apple were as follows: phenolic acids (approximately 25% w/w, mainly chlorogenic acid), phloretin glycosides (approximately 10% w/w, mainly phloridzin), monomeric flavan-3-ols (approximately 15% w/w, catechin), and other compounds (approximately 5% w/w, mainly quercetin glycosides) [1].

2.2 Animals, diets, and experimental protocol

Thirty-two 10-wk-old male Wistar rats were obtained from Japan Clea (Tokyo, Japan); they were maintained under a 12:12-h light–dark cycle. The experimental overview is shown in Fig 1. All the animals were fed laboratory chow (CE7; Clea) for 1 wk. Subsequently, they were divided into two groups – the APP group and the control (CON) group ($N = 16$ in each group). The animals were randomly classified into the two groups, and the sequence of the experiments was random without any bias toward the experimental and CON groups. The composition of the diet in the case of each group is summarized in Table 1 [31]. The APP concentration was determined on the basis of the previous studies in which a significant decrease in adipose tissues and significant increase in muscular strength was

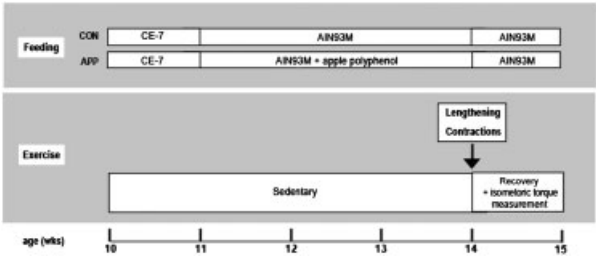

Figure 1. Overview of the study design. Thirty-two 10-wk-old male Wistar rats were randomly grouped into the CON group and the APP group ($N = 16$ in each group). During the first week, the animals were fed CE-2, a commercially available food for animals. In the following 3 wk, the animals were maintained on the formulated diets. AIN-93M is a diet formulated by the American Institute of Nutrition [31]. After the 3-wk dietary control period, all the animals were made to starve for 5 h. Of the 16 animals, 8 were sacrificed with bleeding under anesthesia, and their muscles were harvested and used for mRNA extraction. The torque at the right ankle joint was measured in the remaining eight animals in each group. Subsequently, the animals ($N = 8$ in each group) were subjected to lengthening contractions. After the lengthening contractions, they were fed the control diet, *i.e.* AIN-93M, for 1 wk in order to achieve the same recovery condition. During this 1-wk recovery period, we measured the tetanic torque at the ankle joint.

Table 1. Composition of the diets for the control group and APP group

Ingredients	Diet, g (%)	
	Control	APP
Casein	14.0 (14.0)	14.0 (13.3)
Cornstarch	62.1 (62.1)	62.1 (59.1)
Sucrose	10.0 (10.0)	10.0 (9.5)
Soybean oil	4.0 (4.0)	4.0 (3.8)
Cellulose	5.0 (5.0)	5.0 (4.8)
AIN mineral mixture[a)]	3.5 (3.5)	3.5 (3.3)
AIN vitamin mixture[a)]	1.0 (1.0)	1.0 (0.9)
L-Cystine	0.18 (0.18)	0.18 (0.16)
Choline (bitartrate)	0.25 (0.25)	0.25 (0.23)
t-Butylhydroquinone	0.0008 (0.0008)	0.0008 (0.00076)
APP	0 (0)	5 (4.8)

a) AIN Mineral mixture and AIN vitamin mixture were obtained from Oriental Yeast, Tokyo, Japan.

observed [9, 10]. Animals were maintained on these diets for 3 wk. The food was administered in a dry form. In this 3-wk period, food consumption was measured daily. Since the caloric density of these two diets was the same (*i.e.* 381.2 kcal/100 g), we calculated the energy intake on the basis of the amount of food consumed.

After the 3-wk dietary control period, all the animals were starved for 5 h, and subsequently, 8 out of 16 animals in each group were sacrificed with bleeding under anesthesia, and their muscles were harvested and used for mRNA analysis. Torque measurements were obtained at the right ankle joint for the remaining eigth animals in each group. Subsequently, the animals ($N = 8$ in each group) were subjected to lengthening contractions as shown below. After the lengthening contractions, the animals were fed on a control diet of AIN93M for 1 wk, because we wanted to achieve the same recovery condition. During the 1-wk recovery period, the tetanic torque of the ankle joint was measured. After 1 wk, the animals were sacrificed by bleeding under anesthesia. Immediately after death, the target tissues were harvested. In order to examine the skeletal muscles, both the treated right leg and the untreated left leg were used for further analysis. After measuring the weights, all the specimens were rapidly frozen in liquid N_2 and stored at –80°C until further use.

2.3 Dynamometer setup

The dynamometer setup was essentially the same as that in the previous reports [10, 29, 32]. The torque of the servomotor (RKD514HA; Oriental Motor, Japan) was transmitted to a footplate. The footplate and its angular velocity could be adjusted at 5° intervals. The final deceleration and backlash movement of the footplate was dampened with a magnetic powder brake (ZKB-0.3AN; Mitsubishi Electric, Japan).

The footplate was positioned such that the anatomical axis of the ankle coincided with the axis of the dynamometer shaft. A strain-gauge force transducer (LTB-2KA; Kyowa Electronic Instruments, Japan) was installed to measure the plantar flexion force at the ankle. The angular position of the ankle was measured using a potentiometer (LP06M3R1HA; Murata Manufacturing, Japan). Both the torque and the position signals were sampled at 4000 Hz by using a data acquisition system (PowerLab/16SP; ADInstruments, Australia). We confirmed that this system could be used to evaluate the linear relationship between the torque and its signal, ranging from 1.225 to 2450 mNm.

2.4 Procedure for measuring ankle joint torque in rats

All the animals were anesthetized with the inhalant isoflurane before measuring the torque. The right leg of each animal was used for torque measurements. The procedure for measuring the ankle joint torque was essentially the same as that in the previous reports [10, 29, 32].

The anesthetized rats were firmly secured on a platform in the prone position. Skin electrodes (Vitrode; Nihon Koden, Tokyo, Japan) were affixed on the right medial gastrocnemius, and the muscle was stimulated with pulses of 0.4-ms duration at 10-ms intervals at supramaximal voltage (30 V). The stimulus voltage was adjusted to produce a maximal isometric twitch force in the gastrocnemius and the resultant ankle joint torque was measured. The tetanic torques were measured before and after supplementation of the diet with APP.

2.5 Lengthening contractions for the rat gastrocnemius

Lengthening contractions for the rat gastrocnemius were essentially performed as reported previously with slight modifications [29, 32]. The rats were anesthetized with isoflurane, firmly fixed on the isokinetic dynamometer platform in the prone position, and the gastrocnemius of the right hindlimb was electrically stimulated as previously mentioned. The stimulus voltage was adjusted to produce submaximal isometric twitch torque. The muscle was then stimulated for 3 s to cause a submaximal tetanic contraction. One second after the onset of stimulation, the ankle joint was forcefully isokinetically dorsiflexed to cause an eccentric contraction of the gastrocnemius. The speed and range of the forced lengthening were 240°/s and from 0 to 45°, respectively. The lengthening-contraction exercise consisted of four sessions of five contractions each. The interval between each session was 5 min. The generated torque was measured during the lengthening contractions. To

characterize the change in the isometric tetanic torque during the lengthening contractions, we used the average isometric torque during submaximal isometric contraction at 0° before beginning the forced isokinetic dorsiflexion.

2.6 Measurement of lipid and protein oxidation

The frozen pulverized muscle was homogenized in 50 mM phosphate buffer (pH 7.3) and centrifuged at 10 000 × *g* for 15 min. After centrifugation, the supernatant was used for analysis.

Lipid peroxidation was estimated by measuring the TBARS levels with a commercially available kit (Cayman Chemical, USA). The assay was essentially performed by the TBARS method [33]. Protein peroxidation was estimated by measuring PC levels with a commercially available kit (Cayman Chemical).

2.7 Real-time PCR

Antioxidant protein mRNA levels were evaluated using reverse transcriptase and real-time PCR (RT-PCR). The RNA isolation, reverse transcriptase-PCR, and RT-PCR methods have been previously described. In brief, RNA was extracted (Isogen; Wako Pure Chemicals, Japan) from the untreated left gastrocnemius according to the manufacturer's instructions; thereafter, aliquots of the reverse transcriptase reaction mix (iScript cDNA Synthesis kit; BioRad, USA) were subjected to RT-PCR in a reaction mixture of 25 μL (final volume) that contained a master mix (iQ SYBR Green Supermix; BioRad). The amplification was performed with a Mini Opticon RT-PCR system (BioRad) using the following program: 2 min at 50°C followed by 10 min at 95°C, 15 s at 95°C, and finally 1 min at 60°C for 40 cycles. External standard curves were used for quantitative analysis, and the mRNA levels of each target gene were normalized to those of the control GAPDH gene. We confirmed that the critical threshold (Ct) values of GAPDH were similar between the two groups (APP: Ct = 15.7 ± 1.1, CON: Ct = 15.3 ± 1.2; $N = 6$ in each group; $p > 0.5$). The primers used were 5′-GCAAGCGGTGAACCAGTTGT-3′ (forward primer) and 5′-CAGCCCTTGTGTATTGTCCCCA-3′ (reverse primer) for SOD1 (NM_017050), 5′-CACAAGCACAGCCTCCCT-3′ (forward primer) and 5′-GCGTTAATGTGCGGCTCC-3′ (reverse primer) for SOD3 (NM_017051), 5′-GGTGGCCTTCTTGTTCTGCA-3′ (forward primer) and 5′-GCGTTAATGTGCGGCTCC-3′ (reverse primer) for SOD3 (NM_012880), 5′-GGTGGCCTTCTTGTTCTGCA-3′ (forward primer) and 5′-GCCGGTCTGCTAAGTCGACA-3′ (reverse primer) for CAT (NM_012520), 5′-TGCAATTCACACCTACGTACAGG-3′ (forward primer) and 5′-GCTGATCTTGCCCTTGAGACC-3′ (reverse primer) for GSTα1 (NM_017013), and 5′-CCGTGTTCCTACCCCCAAT-3′ (forward primer) and 5′-ATGTCATCATACTTGGCAGGTTTCT-3′ (reverse primer) for GAPDH (NM_017008) [10, 22]. A comparative Ct method was applied by using standard curves, where the amount of target gene was normalized to that of the endogenous control gene [10, 34, 35].

2.8 Statistical analysis

All the values are presented as the mean ± standard deviation. A two-tailed, unpaired Student's *t*-test was used for statistical comparisons between the two groups. Changes in the tetanic torques were evaluated by a two-way ANOVA with repeated measures and Bonferroni's *post hoc* test. Statistical significance was accepted at the 5% level. Statistical analyses were carried out on a computer running Windows with a statistical software package (SPSS 10.1J; SPSS Japan, Japan).

3 Results

3.1 Characteristics of subjects

No significant differences were observed between the two groups in age, weight, and energy intake, as summarized in Table 2.

3.2 Generated isometric torques during lengthening contractions

A typical example of generated isometric torque during lengthening contractions is shown in Fig 2. The generated torque gradually reduced in both groups. During the lengthening contractions, tetanic torques in the case of the APP group were higher than those in the case of the CON group.

3.3 Isometric torques before and after the lengthening contractions

Before and after the lengthening contractions, the isometric tetanic torque generated by the gastrocnemius was measured. The torques that were measured after the

Table 2. Comparison of the energy intake and body weight of rats between the two groups

	Control	APP
Energy intake (kcal/day/rat)	69.0 ± 13.2	66.9 ± 12.5
Body mass (g)		
Pretreatment	274.1 ± 8.9	274.1 ± 13.0
Posttreatment	364.6 ± 14.8	355.1 ± 18.0

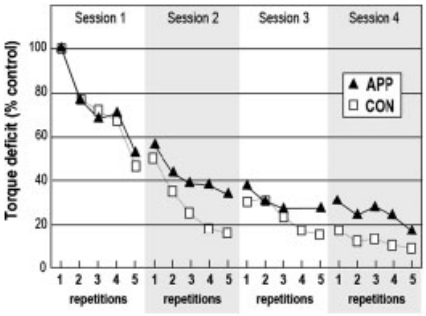

Figure 2. Typical example of generated torques during lengthening contractions. The rats were anesthetized with isoflurane and were firmly secured on the platform of an isokinetic dynamometer in the prone position. Subsequently, the gastrocnemius of the right hind limb was electrically stimulated. The muscle was then stimulated for 3 s to induce a submaximal tetanic contraction. A second after the onset of stimulation, the ankle joint was subjected to forceful isokinetic dorsiflexion to induce an eccentric contraction of the gastrocnemius. The speed and range of the forced lengthening were 240°/s and 0–45°, respectively. The muscle-lengthening contraction exercise consisted of four sessions, each comprising five contractions. The sessions were conducted at 5-min intervals. The generated torque was measured during the lengthening contractions. CON, control group and APP, apple polyphenol group.

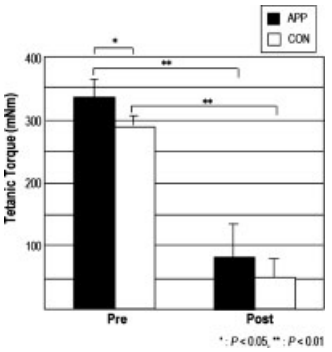

Figure 3. Isometric tetanic torque before and after the lengthening contractions. The anesthetized rats were firmly secured on a platform in the prone position. Skin electrodes were affixed on the right medial gastrocnemius, and the muscle was stimulated with pulses (duration, 0.4 ms) at supramaximal voltage (30 V) and at 10-ms intervals. The resultant ankle joint torque was measured. The tetanic torque was measured before and after the 3-wk diet period. CON group ($N = 8$) and APP group ($N = 8$). Data are shown as mean ± SD.

lengthening contractions were compared with those in the case of the pretreatment control.

Before the lengthening contractions, the generated torque in the case of the APP group was significantly higher than that in the case of the CON group, as reported previously [10]. After the lengthening contractions, force deficits were observed in the two groups. Statistical analysis revealed significant differences between before and after the lengthening contractions ($p < 0.01$) in both groups, as shown in Fig. 3.

3.4 Time-course change in the isometric tetanic torque after the lengthening contractions

The isometric tetanic torque was measured until day 7 after the lengthening contractions. As shown in Fig. 4A, the isometric tetanic forces in the case of the APP group on days 0, 1, 3 ($p < 0.05$), 5, and 7 ($p < 0.01$) were significantly higher than those in the case of the CON group.

The relative force (percent of pretreatment value (%pretreatment value)) was also examined. The %pretreatment value in the case of the APP group was significantly higher than that in the case of the CON group on days 3, 5, and 7 ($p < 0.05$) as shown in Fig. 4B.

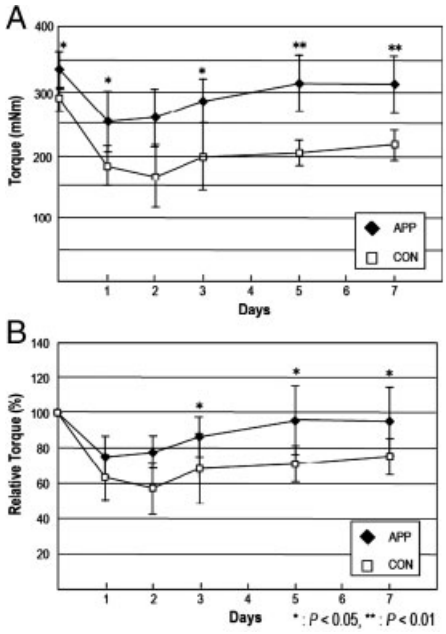

Figure 4. Time-course change of isometric tetanic torque after lengthening contractions. (A) Change in absolute torque after lengthening contractions; (B) Change in relative torque after lengthening contractions. The isometric tetanic torque was measured daily until day 7 after the lengthening contractions. The absolute torque (A) and relative torque to the pretreatment value (B) are indicated. APP group ($N = 8$) and CON group ($N = 8$). Data are shown as mean ± SD.

3.5 Lipid and protein peroxidation

The levels of the oxidized lipid marker of TBARS and the oxidized protein marker of PC in the gastrocnemius were measured (Fig. 5). Both the TBARS and the PC concentrations in the case of the AP group were significantly lower than those in the case of the CON group ($p < 0.05$).

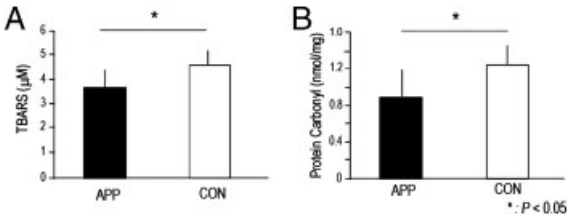

Figure 5. Effect of dietary APPs on TBARS and PC levels on day 7 after lengthening contractions. After the 1-wk recovery period and the induction of the lengthening contractions, the gastrocnemii were harvested and immediately frozen in liquid N_2. The frozen pulverized muscle was homogenized in 50 mM phosphate buffer (pH 7.3), and the supernatant was used for the analysis of TBARS (A) and PC (B). APP group ($N = 8$), CON group ($N = 8$). Data are shown as mean ± SD.

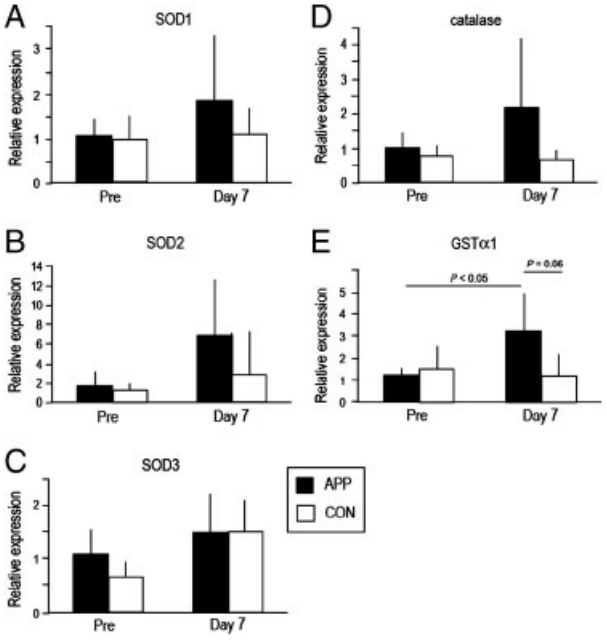

Figure 6. Effect of dietary APPs on the mRNA expression of antioxidative proteins. For 3 wk, the animals were fed the APP diet ($N = 16$) and control diet ($N = 16$). Thereafter, eight animals from each group were sacrificed, and their gastrocnemii were harvested as described in Section 2 (pre). The remaining 16 animals were subjected to lengthening contractions. After a 1-wk recovery period (day 7), the gastrocnemii were harvested from these animals. RNA was extracted from all the 32 gastrocnemii and was used in RT-PCR for mRNA quantification. (A) SOD1, (B) SOD2, (C) SOD3, (D) CAT, and (E) GSTα1, Shaded bar: APP group ($N = 8$ for pre and day 7), open bar: CON group ($N = 8$ for pre and day 7), Data are shown as mean ± SD.

3.6 mRNA expression of antioxidative proteins

We measured the expression of antioxidative proteins (SOD1, SOD2, SOD3, CAT, and GSTα1) in the injured gastrocnemius before and on day 7 after the lengthening contractions (Fig 6). RT-PCR analysis revealed that in the APP group, GSTα1 expression on day 7 was significantly higher than that at pretreatment ($p < 0.05$). On day 7, the GSTα1 expression in the case of the APP group showed a higher trend than that in the case of the CON group ($p = 0.06$). Although this difference was not statistical significant, the mRNA expression levels of other antioxidants in the APP group also showed an increasing trend on day 7 after the lengthening contractions.

4 Discussion

The major finding in this study was that the APP group exhibited a significantly lower force deficit and earlier recovery after the strenuous lengthening contractions. It is well known that polyphenols possess antioxidative properties [3, 4, 8]. Furthermore, we confirmed that the levels of serum TBARS and PC, the major indicators of oxidative damage, in the case of the APP group were significantly lower than that in the case of the CON group. Analysis of the mRNA expression of antioxidative proteins showed that GSTα1 expression was significantly higher in the case of the APP group than that in the case of the CON group, suggesting that the mice in the APP group possessed a higher antioxidative ability than those in the CON group.

In previous studies, the effect of dietary polyphenols on isometric tetanic torque or muscle strength after lengthening contractions was not examined. In this study, we showed that the force deficits in the case of the APP group were significantly lower than those in the case of the CON group. Since we confirmed that there were no significant differences in the force deficits measured immediately after the lengthening contractions between the groups, the initial mechanical disorders are probably similar in both groups. Thus, the subsequent inflammation and/or delayed recovery should cause duration of force deficit. As discussed below, we believe that dietary polyphenols and other antioxidative proteins scavenge reactive oxygen species, thereby reducing muscle injuries.

As mentioned in Section 1, dietary polyphenols reportedly reduced the levels of malondialdehyde, PC [30], 4-hydroxy-2-nonenal-modified protein, and 8-hydroxy-2′-deoxyguanosine [23, 24] induced by strenuous exercise, indicating that dietary polyphenols have a protective effect against exercise-induced oxidative stress. In this study, we confirmed that the serum TBARS and PC levels in the case of the APP group were significantly lower than those in the case of the CON group. Above all, we consider that dietary APPs have beneficial antioxidant properties against strenuous exercise, similar to those in the case of other polyphenols. A straightforward explanation for these antioxidative properties could be that the polyphenols scavenge the reactive oxygen species that cause lipid, protein, and DNA damage. The mRNA expression levels of antioxidative proteins showed an increasing trend, but the increase was not significant.

In this study, we also confirmed that the expression of GSTα1, one of the antioxidative proteins, was significantly higher in the APP-treated group. Glutathione participates in detoxification at several different levels, and may scavenge free radicals, reduce peroxide, or form conjugates with electrophilic compounds. GSTα acts as a glutathione peroxidase and plays a protective role against xenobiotics. GSTα can reduce lipid hydroperoxides. Nijhoff and Peters have demonstrated that dietary polyphenol enhances GST detoxification by inducing the expression of GSTα and GST isoenzymes in rat [36]. Hofmann *et al.*, also showed that dietary polyphenols induced delayed enhancement of glutathione-*S*-transferase P1 in human leukocytes *in vivo* [37]. These results indicate that dietary polyphenols enhance the expression of GSTs. GSTs catalyze the reaction of a wide range of electrophiles with glutathione. Not only polyphenols but also elevated levels of GSTs reduce tissue damage. Further, examining the expression and activity of enzymes involved in glutathione metabolism, such as glutathione peroxidase and glutaredoxin, may shed light on the relationship between APPs and glutathione detoxification.

In this study, we focused only on the antioxidative effect of APPs. It is worth noting that the physiological outcome and antioxidative properties are not directly related. Although it is important to consider the antioxidative property in relation to this phenomenon, we would like to discuss other possible mechanisms of preventing muscle injury with APPs. In 2005, Lagouge *et al.* showed that resveratrol activates peroxisome proliferator-activated receptor coactivator (PGC-1α *via* sirtuin 1 [38]. PGC-1α plays a role in fast-to-slow fiber-type conversion [39]. On the other hand, severe lengthening contraction injuries, namely, strain injury, frequently occurred in the hamstring, which is rich in fast-twitch fibers [40]. These studies indicate that that fast-to-slow fiber transition probably occurs in the muscles of animals fed with APP.

Another possibility is related to the mitogen-activated protein kinase (MAPK) pathway. Several researchers have shown that polyphenols inhibit p38 MAPK activity. Further, it is known that p38 MAPK is involved in apoptosis and stress response [41–43]. It has been observed that in a skeletal muscle injury, p38 MAPK pathways are transiently upregulated *via* the tumor necrosis factor-α in C2C12 cells [44], the human vastus lateralis muscle [45], and mouse soleus muscle [46, 47]. Although p38 MAPK activation is required for muscle regeneration [47], experimentally generated excessive amounts of tumor necrosis factor-α inhibited the recovery process [48]. In the lengthening contraction model, the response to excessive amounts of p38 MAPK may be suppressed by APP; this in turn may facilitate early recovery.

In summary, we conclude that dietary APPs have a preventive effect against lengthening contraction-induced muscle injuries. The antioxidative effects of polyphenols and elevated antioxidative proteins provide protection against further damage after injurious muscle contractions.

The authors have declared no conflict of interest.

5 References

[1] Kanda, T., Akiyama, H., Yanagida, A., Tanabe, M. *et al.*, Inhibitory effects of apple polyphenol on induced histamine release from RBL-2H3 cells and rat mast cells. *Biosci. Biotechnol. Biochem.* 1998, *62*, 1284–1289.

[2] Ohnishi-Kameyama, M., Yanagida, A., Kanda, T., Nagata, T., Identification of catechin oligomers from apple (*Malus pumila* cv. Fuji) in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and fast-atom bombardment mass spectrometry. *Rapid Commun. Mass Spectrom.* 1997, *11*, 31–36.

[3] Eberhardt, M. V., Lee, C. Y., Liu, R. H., Antioxidant activity of fresh apples. *Nature* 2000, *405*, 903–904.

[4] Lee, K. W., Kim, Y. J., Kim, D. O., Lee, H. J., Lee, C. Y., Major phenolics in apple and their contribution to the total antioxidant capacity. *J. Agric. Food Chem.* 2003, *51*, 6516–6520.

[5] Pearson, D. A., Tan, C. H., German, J. B., Davis, P. A., Gershwin, M. E., Apple juice inhibits human low density lipoprotein oxidation. *Life Sci.* 1999, *64*, 1913–1920.

[6] Akiyama, H., Sakushima, J., Taniuchi, S., Kanda, T. *et al.*, Antiallergic effect of apple polyphenols on the allergic model mouse. *Biol. Pharm. Bull.* 2000, *23*, 1370–1373.

[7] Aprikian, O., Duclos, V., Guyot, S., Besson, C. *et al.*, Apple pectin and a polyphenol-rich apple concentrate are more effective together than separately on cecal fermentations and plasma lipids in rats. *J. Nutr.* 2003, *133*, 1860–1865.

[8] Shoji, T., Kobori, M., Shinmoto, H., Tanabe, M., Tsushida, T., Progressive effects of phloridzin on melanogenesis in B16 mouse melanoma cells. *Biosci. Biotechnol. Biochem.* 1997, *61*, 1963–1967.

[9] Nakazato, K., Song, H., Waga, T., Effects of dietary apple polyphenol on adipose tissues weights in Wistar rats. *Exp. Anim.* 2006, *55*, 383–389.

[10] Nakazato, K., Song, H., Waga, T., Dietary apple polyphenols enhance gastrocnemius function in Wistar rats. *Med. Sci. Sports Exerc.* 2007, *39*, 934–940.

[11] Buetler, T. M., Renard, M., Offord, E. A., Schneider, H., Ruegg, U. T., Green tea extract decreases muscle necrosis in mdx mice and protects against reactive oxygen species. *Am. J. Clin. Nutr.* 2002, *75*, 749–753.

[12] Dorchies, O. M., Wagner, S., Vuadens, O., Waldhauser, K. *et al.*, Green tea extract and its major polyphenol (-)-epigallocatechin gallate improve muscle function in a mouse model for Duchenne muscular dystrophy. *Am. J. Physiol. Cell Physiol.* 2006, *290*, C616–C625.

[13] Ji, L. L., Antioxidants and oxidative stress in exercise. *Proc. Soc. Exp. Biol. Med.* 1999, *222*, 283–292.

[14] Cooper, C. E., Vollaard, N. B., Choueiri, T., Wilson, M. T., Exercise, free radicals and oxidative stress. *Biochem. Soc. Trans.* 2002, *30*, 280–285.

[15] Aoi, W., Naito, Y., Takanami, Y., Kawai, Y. *et al.*, Oxidative stress and delayed-onset muscle damage after exercise. *Free Radic. Biol. Med.* 2004, *37*, 480–487.

[16] Uchiyama, S., Tsukamoto, H., Yoshimura, S., Tamaki, T., Relationship between oxidative stress in muscle tissue and

weight-lifting-induced muscle damage. *Pflugers Arch.* 2006, *452*, 109–116.

[17] Bloomer, R. J., Goldfarb, A. H., Wideman, L., McKenzie, M. J., Consitt, L. A., Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *J. Strength Cond. Res.* 2005, *19*, 276–285.

[18] Bloomer, R. J., Falvo, M. J., Fry, A. C., Schilling, B. K. *et al.*, Oxidative stress response in trained men following repeated squats or sprints. *Med. Sci. Sports Exerc.* 2006, *38*, 1436–1442.

[19] Liu, J., Yeo, H. C., Overvik-Douki, E., Hagen, T. *et al.*, Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J. Appl. Physiol.* 2000, *89*, 21–28.

[20] Paula, F. B., Gouvea, C. M., Alfredo, P. P., Salgado, I., Protective action of a hexane crude extract of *Pterodon emarginatus* fruits against oxidative and nitrosative stress induced by acute exercise in rats. *Biomed Chromatogr. Complement Altern. Med.* 2005, *5*, 17.

[21] Aoi, W., Naito, Y., Yoshikawa, T., Exercise and functional foods. *Nutr. J.* 2006, *5*, 15.

[22] Aoi, W., Naito, Y., Nakamura, T., Akagiri, S. *et al.*, Inhibitory effect of fermented milk on delayed-onset muscle damage after exercise. *J. Nutr. Biochem.* 2007, *18*, 140–145.

[23] Aoi, W., Naito, Y., Sakuma, K., Kuchide, M. *et al.*, Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice. *Antioxid. Redox Signal.* 2003, *5*, 139–144.

[24] Kato, Y., Miyake, Y., Yamamoto, K., Shimomura, Y. *et al.*, Preparation of a monoclonal antibody to N(epsilon)-(Hexanonyl)lysine: application to the evaluation of protective effects of flavonoid supplementation against exercise-induced oxidative stress in rat skeletal muscle. *Biochem. Biophys. Res. Commun.* 2000, *274*, 389–393.

[25] Bloomer, R. J., Goldfarb, A. H., McKenzie, M. J., Oxidative stress response to aerobic exercise: comparison of antioxidant supplements. *Med. Sci. Sports Exerc.* 2006, *38*, 1098–1105.

[26] Goldfarb, A. H., Patrick, S. W., Bryer, S., You, T., Vitamin C supplementation affects oxidative-stress blood markers in response to a 30-minute run at 75% VO2max. *Int. J. Sport Nutr. Exerc. Metab.* 2005, *15*, 279–290.

[27] Goldfarb, A. H., Bloomer, R. J., McKenzie, M. J., Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise. *Med. Sci. Sports Exerc.* 2005, *37*, 234–239.

[28] Bryer, S. C., Goldfarb, A. H., Effect of high dose vitamin C supplementation on muscle soreness, damage, function, and oxidative stress to eccentric exercise. *Int. J. Sport Nutr. Exerc. Metab.* 2006, *16*, 270–280.

[29] Song, H., Nakazato, K., Nakajima, H., Effect of increased excursion of the ankle on the severity of acute eccentric contraction-induced strain injury in the gastrocnemius: an *in vivo* rat study. *Am. J. Sports Med.* 2004, *32*, 1263–1269.

[30] You, T., Goldfarb, A. H., Bloomer, R. J., Nguyen, L. *et al.*, Oxidative stress response in normal and antioxidant supplemented rats to a downhill run: changes in blood and skeletal muscles. *Can. J. Appl. Physiol.* 2005, *30*, 677–689.

[31] Reeves, P. G., Nielsen, F. H., Fahey, G. C., Jr., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* 1993, *123*, 1939–1951.

[32] Ochi, E., Nakazato, K., Ishii, N., Effects of eccentric exercise on joint stiffness and muscle connectin (titin) isoform in the rat hindlimb. *J. Physiol. Sci.* 2007, *57*, 1–6.

[33] Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *J. Agri. Food Chem.* 1994, *42*, 1931–1937.

[34] Wong, M. L., Medrano, J. F., Real-time PCR for mRNA quantitation. *Biotechniques* 2005, *39*, 75–85.

[35] Kramer, D. K., Ahlsen, M., Norrbom, J., Jansson, E. *et al.*, Human skeletal muscle fibre type variations correlate with PPAR alpha, PPAR delta and PGC-1 alpha mRNA. *Acta. Physiol. (Oxf)* 2006, *188*, 207–216.

[36] Nijhoff, W. A., Bosboom, M. A., Smidt, M. H., Peters, W. H., Enhancement of rat hepatic and gastrointestinal glutathione and glutathione S-transferases by alpha-angelicalactone and flavone. *Carcinogenesis* 1995, *16*, 607–612.

[37] Hofmann, T., Liegibel, U., Winterhalter, P., Bub, A. *et al.*, Intervention with polyphenol-rich fruit juices results in an elevation of glutathione S-transferase P1 (hGSTP1) protein expression in human leucocytes of healthy volunteers. *Mol. Nutr. Food Res.* 2006, *50*, 1191–1200.

[38] Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H. *et al.*, Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 2006, *127*, 1109–1122.

[39] Lin, J., Wu, H., Tarr, P. T., Zhang, C. Y. *et al.*, Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature* 2002, *418*, 797–801.

[40] Petersen, J., Holmich, P., Evidence based prevention of hamstring injuries in sport. *Br. J. Sports Med.* 2005, *39*, 319–323.

[41] Yang, Y. T., Weng, C. J., Ho, C. T., Yen, G. C., Resveratrol analog-3,5,4′-trimethoxy-trans-stilbene inhibits invasion of human lung adenocarcinoma cells by suppressing the MAPK pathway and decreasing matrix metalloproteinase-2 expression. *Mol. Nutr. Food Res.* 2009, *53*, 407–416.

[42] Schoene, N. W., Kelly, M. A., Polansky, M. M., Anderson, R. A., A polyphenol mixture from cinnamon targets p38 MAP kinase-regulated signaling pathways to produce G2/M arrest. *J. Nutr. Biochem.* 2009, *20*, 614–620.

[43] Guo, S., Lu, J., Subramanian, A., Sonenshein, G. E., Microarray-assisted pathway analysis identifies mitogen-activated protein kinase signaling as a mediator of resistance to the green tea polyphenol epigallocatechin 3-gallate in her-2/neu-overexpressing breast cancer cells. *Cancer Res.* 2006, *66*, 5322–5329.

[44] Yeow, K., Cabane, C., Turchi, L., Ponzio, G., Derijard, B., Increased MAPK signaling during *in vitro* muscle wounding. *Biochem. Biophys. Res. Commun.* 2002, *293*, 112–119.

[45] Aronson, D., Wojtaszewski, J. F., Thorell, A., Nygren, J. *et al.*, Extracellular-regulated protein kinase cascades are activated in response to injury in human skeletal muscle. *Am. J. Physiol.* 1998, *275*, C555–C561.

[46] Chen, S. E., Gerken, E., Zhang, Y., Zhan, M. *et al.*, Role of TNF-{alpha} signaling in regeneration of cardiotoxin-injured muscle. *Am. J. Physiol. Cell Physiol.* 2005, *289*, C1179–C1187.

[47] Chen, S. E., Jin, B., Li, Y. P., TNF-alpha regulates myogenesis and muscle regeneration by activating p38 MAPK. *Am. J. Physiol. Cell Physiol.* 2007, *292*, C1660–C1671.

[48] Langen, R. C., Schols, A. M., Kelders, M. C., van der Velden, J. L. *et al.*, Muscle wasting and impaired muscle regeneration in a murine model of chronic pulmonary inflammation. *Am. J. Respir. Cell Mol. Biol.* 2006, *35*, 689–696.

