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Effect of Dietary Nitrate on Force Production and Sarcoplasmic Reticulum Ca^{2+} Handling in Rat Fast-Twitch Muscles Following Eccentric Contraction

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Abstract

Impaired excitation-contraction coupling occurs in eccentric contraction (ECC)-induced damaged muscles. It has been suggested that sarcoplasmic reticulum (SR) is susceptible to damage in the overstretched regions possibly marking the basis of excitation-contraction coupling damage. Recent studies have shown that dietary nitrate supplementation enhances SR function in fast-twitch muscles. In this study, we aimed to investigate whether dietary nitrate supplementation can alleviate a decline in muscle contractile properties and SR function following ECC. To this end, force production, Ca^{2+} uptake, Ca^{2+} release, and Ca^{2+}-ATPase activity of the SR were examined in rat fast-twitch muscles immediately following ECC for 200 repetitions. In comparison with contralateral resting muscles, nitrate supplementation for up to 3 days resulted in an obvious decline in force production. However, there were no differences in terms of force production between 6-day nitrate-treated and contralateral muscles. Similar to the observations regarding force production, the SR Ca^{2+} release rate changed from an obvious decrease following the 0- and 3-day dietary nitrate supplementation to no difference following the 6-day nitrate supplementation. In contrast, ECC decreased the Ca^{2+}-ATPase activity and Ca^{2+} uptake rate, irrespective of the period of dietary nitrate supplementation. Overall, these results indicate that dietary nitrate supplementation can alleviate ECC-related decreases in force production mediated through inhibited reductions in the SR Ca^{2+} release function.

Keywords

Supplementation, Ca^{2+}-ATPase Activity, Ca^{2+} Uptake, Ca^{2+} Release

1. Introduction

Fatigue-induced changes in force production can be analyzed in terms of a generally decreased ability of cross-bridges formation to generate force, decreased myofibrillar Ca^{2+} sensitivity, decreased sarcoplasmic reticulum (SR) Ca^{2+} release, or combination of these [1]. Previous studies that used skinned or intact fibers indicated superoxide-dependent protein modifications [2], glycogen depression [3] [4], and protein degradation [5] as the probable causes of decreased SR Ca^{2+} release [6].

Unaccustomed eccentric contraction (ECC) induces skeletal muscle damage characterized by a long-lasting decrease in muscle strength and delayed onset muscle soreness [7]. Muscles damaged by unaccustomed ECC are characterized by sarcolemmal disruption and myofibrillar disorganization during ECC [8] and crystallized structures within the Z disk and SR swelling immediately following ECC [9]; consequently, overstretched sarcomeres act as the origin of such muscle damage [10]. Reportedly, impaired excitation-contraction coupling occurs in eccentrically damaged mammalian muscles [8]. It has been suggested that sarcomeres within myofibrils, transverse tubules (t-tubules), and SR are susceptible to damage in the overstretched regions, possibly marking the basis of excitation-contraction coupling damage [11].

Recently, the presence of nitrates within the diet and their potential as a source of nitric oxide (NO) has gained increasing attention. NO itself plays an important regulatory role in several physiological processes, such as vasodilatation, blood pressure regulation, mitochondrial respiration, cell signaling, and mitochondrial biogenesis [12] [13] [14]. The classic mechanism for NO generation via the oxidation of L-arginine in a reaction catalyzed by nitric oxide synthase (NOS) has been well-documented [15].

Dietary supplementation with inorganic nitrate an NO donor, enhances NO bioavailability, reduces oxygen cost of exercise, and increases exercise performance in endurance exercise [16]. Further, supplemented nitrate enhances endurance performance [17] and reduces the PCr cost of force production [18]. Removal of dietary nitrate supplementation from the diet has been shown to reduce running distance and speed to the control level, despite the gain of improved endurance during dietary nitrate supplementation [19]. Recent studies have shown that dietary nitrate supplementation enhances SR Ca^{2+} release in mouse fast-twitch muscles [20] and that NO, synthesized from L-arginine injection, increases Ca^{2+} regulatory protein concentrations [21].

Based on these findings, we designed a hypothesis that dietary nitrate supplementation inhibits ECC-induced alterations in the SR function. The main objec-

tive of the present study was to examine whether dietary nitrate supplementation prior to ECC would alleviate the decline in muscle contractile properties and SR Ca^{2+} handling in rat fast-twitch skeletal muscle immediately following ECC.

2. Materials and Methods

2.1. Animal Care and Nitrate Ingestion

Thirty 9-wk-old male Wistar rats were housed in a thermally controlled room maintained between 20˚C and 24˚C under a 12-h light/dark cycle. Water and food were provided *ad libitum*. All study procedures were approved by the Animal Care Committee of Hiroshima University. The rats were randomly divided into three nitrate-treated groups (n = 10 for each group) and were administered dietary nitrate supplementation for 0 (non-treated), 3, and 6 days. The period of dietary nitrate supplementation used in this study is similar to that utilized in the study by Hernández *et al.* [20]. The rats were administered 1 mmol kg^{-1} day^{-1} $NaNO_3$ diluted with 4 mL tap water. We used the nitrate dose described by Ferguson *et al.* [22].

2.2. Exercise Procedures

Throughout the experiment, the rats were deeply anesthetized with an intraperitoneal injection of a mixture of medetomidine (0.4 mg kg body wt^{-1}), midazolam (2.0 mg kg body wt^{-1}), and butorphanol (2.5 mg kg body wt^{-1}). ECC was performed as described previously [11]. Briefly, an animal was placed in the supine position on a supporting platform, with the left foot secured in a foot holder attached to the rim of a servomotor. Further, the knee was secured using a strap such that the foot was positioned perpendicular to the lower leg. A pair of sterilized needle electrodes was inserted through aseptically prepared skin to stimulation of the peroneal nerve in the left leg that innervates the left extensor digitorum longus (EDL) and tibialis anterior (TA) muscles. The correct location of the needles was confirmed by the dorsiflexion of the ankle joints and extension of the toes in response to the electrical stimulation of the common peroneal nerve. Repetitive contractions of the EDL and TA muscles were induced by electrical stimuli applied to the common peroneal nerve. In addition, muscle contractions were elicited by stimulating the peroneal nerve using a 1000-ms train of 1-ms pulse at 50 Hz and supramaximal voltage. For the ECC protocol, the experimental leg was forcibly extended with the servomotor at an angular velocity of 150˚ s^{-1} from the ankle joint, from 30˚ to 180˚, in synchrony with the electrical stimulation of the nerve over a 1-s period. The ECC was repeated every 4 s for a total of 200 repetitions.

Immediately following ECC, the experimental EDL and TA muscles (left hindlimb) as well as contralateral resting muscles (right hindlimb) were quickly excised. The amount of EDL or TA muscle obtained was considered too small for physiological or biochemical analysis. Therefore, EDL and TA muscles were used to measure the force production and biochemical analyses, respectively. As

reported by Kanzaki *et al.* [23], EDL and TA muscles have almost the same composition of rat fast-twitch fibers and exhibit similar functional deficits following ECC. Some previous studies assumed that these muscles are similarly affected by ECC [24]. At the end of the experiments, the rats were euthanized with pentobarbital sodium (200 mg kg body wt^{-1}), followed by cervical dislocation.

2.3. Measurement of Isometric Force Production

Isometric force production of the EDL muscles was recorded at 30˚C in a chamber filled with a solution of the following composition (as previously described [24]): 115 mM NaCl, 5 mM $KHCO_3$, 1 mM $MgCl_2$, 20 mM $NaHCO_3$, 2 mM $CaCl_2$, 5 mM *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, 11 mM glucose, 0.3 mM glutamic acid, and 0.38 mM glutamine. The solution was continuously bubbled with 95% O_2/5% CO_2, yielding a pH of 7.4. The EDL muscles were connected to an isometric force transducer. The stimulation pulses were applied via two platinum plate electrodes placed on each side of the muscle. The muscles were allowed to equilibrate for 10 min, during which, the optimal length was determined. Tetanic forces were elicited via direct stimulation at 20, 40, 60, and 80 Hz using a supramaximal voltage, 1-ms pulses, and 1.5-s trains. Force was recorded on a personal computer, analyzed using dedicated software (Lab Chart; ADInstruments, Nagoya, Japan), and normalized to the cross-sectional area, where the cross-sectional area was computed as the muscle wet weight divided by the product of the muscle length and density (1.07 g mL^{-1}).

2.4. Homogenate Preparation

The TA muscle pieces were diluted in a ratio of 1:9 (mass vol^{-1}) in ice-cold homogenizing buffer (pH 7.4) composed of 300 mM sucrose, 20 mM MOPS/KOH, 0.0014 mM pepstatin, 0.83 mM benzamidine, 0.0022 mM leupeptin, and 0.2 mM phenylmethanesulfonyl fluoride [11]. They were mechanically homogenized thrice with a hand-held glass homogenizer (Asone, Osaka, Japan) at 5000 rpm for 30-s bursts separated by 30-s breaks. Then, the homogenate was centrifuged at 5000× *g* for 10 min. The obtained supernatant was quickly frozen in liquid nitrogen and stored at −80˚C. The measurements of SR Ca^{2+}-ATPase activity and Ca^{2+}-uptake and -release rate were performed using the supernatant. The protein concentrations were determined using the method described by Bradford [25].

2.5. SR Ca^{2+}-ATPase Activity

The SR Ca^{2+}-ATPase activity in the presence of 1 μg mL^{-1} Ca^{2+} ionophore A23187 (Sigma) was spectrophotometrically measured in muscle homogenates in triplicate at 37˚C as per the methods described by Simonides & van Hardeveld [26]. The assay mixture (pH 7.1) comprised 20 mM *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid, 1 mM EGTA, 200 mM KCl, 15 mM $MgCl_2$, 0.8 mM $CaCl_2$, 10 mM sodium azide (NaN_3), 0.4 mM NADH, 10 mM phosphoenolpyruvate, 12.1 U mL^{-1} pyruvate kinase, and 20.2 U

mL^{-1} lactate dehydrogenase. The reaction was initiated by adding Mg-ATP at a final concentration of 4 mM. Finally, the $CaCl_2$ concentration was increased to 20 mM to selectively inhibit SR Ca^{2+}-ATPase activity. The remaining activity was considered as the background ATPase activity. The activity of SR Ca^{2+}-ATPase was calculated as the difference between the total and background ATPase activities.

2.6. SR Ca^{2+} Uptake and Release Rate

SR Ca^{2+} uptake and release rates were measured at 37˚C in triplicate using the Ca^{2+} fluorescent dye indo-1, as previously described [24]. Aliquots of the homogenate were incubated for 3 min at 37˚C in an assay buffer (pH 7.0) composed of 100 mM KCl, 20 mM N-2-hydroxyethylpiperazine-N"-2-ethanesulfonic acid, 10 mM NaN_3, 6.8 mM potassium oxalate, 0.5 mM $MgCl_2$ and 0.001 mM indo-1. SR Ca^{2+} uptake was initiated by the addition of 1 mM Mg-ATP, which was continued until little or no change in the Ca^{2+} concentration ($[Ca^{2+}]$) was observed. Then, Ca^{2+} release was initiated by adding 10 mM 4-chloro-m-cresol. $[Ca^{2+}]$ was monitored using a spectrofluorometer (FB-8300ST; Nihon-Bunko, Tokyo, Japan) and computed as per the ratiometric method [27].

2.7. Statistical Analysis

Statistical analyses were conducted using the SigmaPlot statistical software (version 14; Systat Software, San Jose, CA). All data are presented as mean ± standard error values of the mean (SE) values. Two-way ANOVA was used to investigate the effects of the contractile protocol (ECC vs. Rest) and period of dietary nitrate supplementation. When significant differences were detected, Holm-Sidak post hoc test was performed. Statistical significance was set at P-value < 0.05.

3. Results

3.1. Isometric Force Production

There were no significant differences among the measurement results of the three resting groups. ECC-induced force production by 80-Hz stimulation in the 0-, 3-, and 6-day nitrate-treated muscles declined to 71.9%, 73.9%, and 83.5%, respectively, compared with that in the contralateral resting muscles (**Figure 1**). Regarding force production, compared with the contralateral resting muscles, the 0- and 3-day nitrate-treated muscles showed a significant decline ($P = 0.005$ and $P = 0.001$, respectively), whereas the 6-day nitrate-treated muscles showed no significant difference ($P = 0.125$).

3.2. SR Ca^{2+} Handling Function

The Ca^{2+}-ATPase activity following ECC in the 0-, 3-, and 6-day nitrate-treated muscles was 86.8%, 97.6%, and 96.5%, respectively, compared with that of in the contralateral resting muscles (**Figure 2**). Further, the rate of Ca^{2+} uptake was 90.5%, 92.3%, and 93.2% in the 0-, 3-, and 6-day nitrate-treated muscles, respectively, compared with that in the contralateral resting muscles (**Figure 3**).

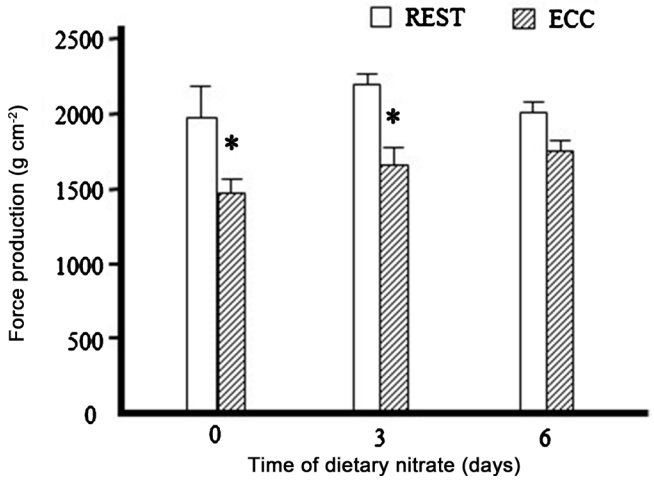

Figure 1. Effect of dietary nitrate on force production immediately following eccentric contraction. The rats were orally administered nitrate (1 mmol kg^{-1} day^{-1}) prior to the ECC protocol. ECC was repeated in the anterior muscles of the left hind–limb for a total of 200 cycles. The rested muscles of the contralateral (right) legs were used as controls. Immediately following ECC, the extensor digitorum longus muscles were excised and used to measure of isometric force production. Isometric forces were evoked via direct electrical stimulation at 80 Hz. The values represent mean ± standard error of mean values (SE) (n = 8 for each muscle). *P < 0.05, versus rested muscle within rats. ECC, eccentric contraction.

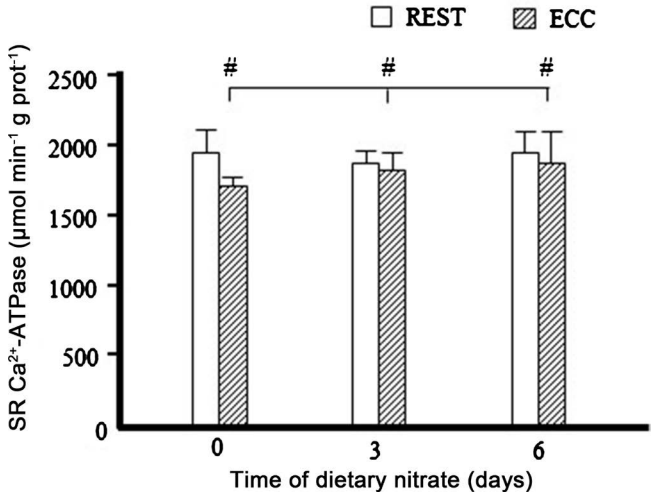

Figure 2. Effect of dietary nitrate on SR Ca^{2+}-ATPase activity immediately following eccentric contraction. The rats were orally administered nitrate (1 mmol kg^{-1} day^{-1}) prior to the ECC protocol. Immediately following ECC of a total of 200 cycles, the tibialis anterior muscles were excised. Activities were measured on muscle homogenates. The values represent mean ± standard error of mean values (SE) (n = 8 for each muscle). #P < 0.05, significant main effect for ECC (rest > ECC). ECC, eccentric contraction; SR, sarcoplasmic reticulum.

Regarding Ca^{2+}-ATPase activity and Ca^{2+} uptake, a main effect was observed between ECC and Rest (Rest > ECC: P = 0.047 and P = 0.036, respectively).

Although the rate of ECC-induced Ca^{2+} release significantly declined to 78.6% and 77.6% in the 0- and 3-day nitrate-treated muscles, respectively, compared

with that in the contralateral resting muscles (Figure 4, $P = 0.036$ and $P = 0.022$, respectively), no significant difference in this regard was found between the 6-day nitrate-treated and contralateral resting muscles (102.9%, $P = 0.797$).

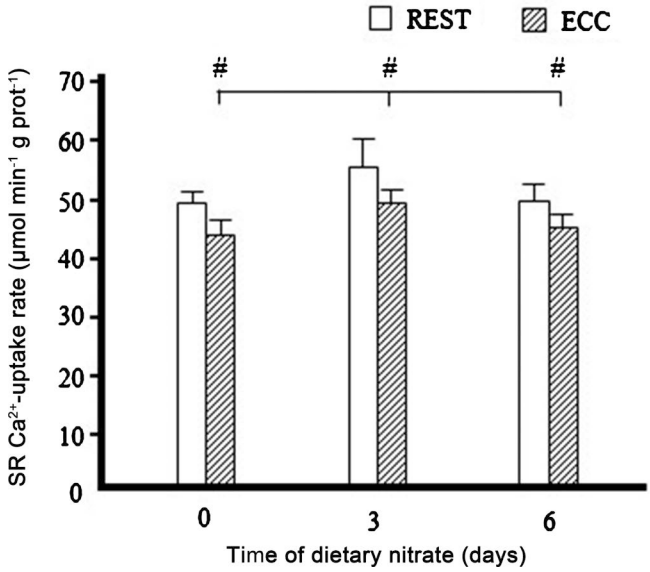

Figure 3. Effect of dietary nitrate on SR Ca^{2+}-uptake rate immediately following eccentric contraction. The rats were orally administered nitrate (1 mmol kg^{-1} day^{-1}) prior to the ECC protocol. Immediately following ECC of a total of 200, the tibialis anterior muscles were excised. SR Ca^{2+}-uptake rate was measured on muscle homogenates. The values represent mean ± standard error of mean values (SE) ($n = 8$ for each muscle). $^{\#}P < 0.05$, significant main effect for ECC (rest > ECC). ECC, eccentric contraction; SR, sarcoplasmic reticulum.

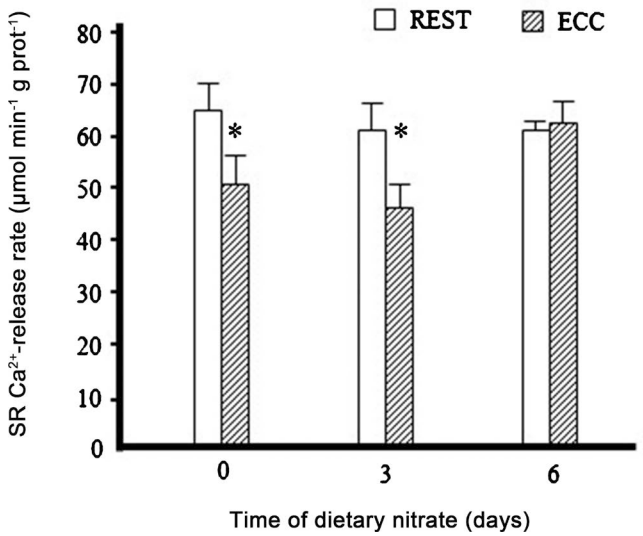

Figure 4. Effect of dietary nitrate on SR Ca^{2+}-release rate immediately following eccentric contraction. The rats were orally administered nitrate (1 mmol kg^{-1} day^{-1}) prior to the ECC protocol. Immediately following ECC of a total of 200, the tibialis anterior muscles were excised. SR Ca^{2+}-release rate was measured on muscle homogenates. The values represent mean ± standard error of mean values (SE). $^{*}P < 0.05$, versus rested muscle within rats. ECC, eccentric contraction; SR, sarcoplasmic reticulum.

4. Discussion

Dietary nitrate supplementation has been shown to exert a variety of effects on physiological function, with recent evidence that short-term supplementation lowers the resting blood pressure [28] [29] [30], reduces the energetic cost of exercise [28] [30] [31], activates muscle contraction [20] [32] [33], and enhances endurance [17] [34] and intense intermittent exercise performance [35]. However, to the best of our knowledge, there is no sufficient evidence regarding the effects of nitrate ingestion before ECC on muscle contractile properties. The following remarkable results were noted in this study. First, 6-day dietary nitrate supplementation, but not 3-day supplementation, mitigated ECC induced decreases in force production, demonstrating that 6-day dietary nitrate supplementation prior to the ECC protocol markedly improved force production in rat fast-twitch muscles following ECC.

ECC results in an inability to produce the desired force characterized by triad deformation [36], sarcomere inhomogeneity [37], increased membrane permeability [38], inflammation [39], and proteolysis [21] [40]. It has been well documented that modified intracellular Ca^{2+} handling induced by dietary nitrate supplementation may enhance muscle performance [31] [41]. Recently, an enhancement in SR Ca^{2+} handling function by dietary nitrate supplementation in mouse fast-twitch muscle was reported [20]. Second, 6-day nitrate supplementation prior to the ECC protocol inhibited ECC-induced decreases in the SR Ca^{2+} release rate in rat fast-twitch muscles. We noted that loss of ECC-induced contractile activity can be attributed to a failure of SR Ca^{2+} release [11]. Nitrate ingestion enhanced SR Ca^{2+} release and tetanic force production via modifications of the cellular Ca^{2+} handling components in mouse fast-twitch muscle [20]. NO is primarily synthesized from L-arginine by neuronal NOS and can enhance Ca^{2+} regulatory proteins concentration [15] [21]. In the skeletal muscles, L-arginine-driven NO is moderately generated in the resting state, and its production markedly increases with contractile activity [15]. Considering these observations, it can be hypothesized that enhanced SR Ca^{2+} release following dietary nitrate supplementation can alleviate ECC-induced loss of contractile function.

Ca^{2+}-regulated cysteine proteases (calpains) comprise a proteolytic system in the skeletal muscles. Previous *in vitro* studies on the effect of NO on calpains demonstrated that the use of NO donors can inhibit the activation of calpains mediated via S-nitrosylation [42]. In a recent study, it was demonstrated that treatment with a calpain inhibitor could attenuate ECC-elicited force deficits, proteolysis of proteins regulated Ca^{2+} release from SR in fast-twitch muscles of rats [43], and L-arginine ingestion can attenuate ECC-induced proteolysis of Ca^{2+} regulatory proteins by decreasing calpain activation via S-nitrosylation [21]. Considering these findings, it was suggested that the attenuation of the ECC induced decline of SR Ca^{2+} release in this study is attributable to the decreased calpain activation via S-nitrosylation induced by the 6-day ingestion of nitrate,

an NO donor, although calpain was not analyzed in this study. Thus effects dietary nitrate supplementation on contractile function and calpain activity following ECC should be explored in future investigations.

5. Conclusion

In conclusion, the present results indicated that nitrate ingestion is capable of alleviating ECC-related decreases in muscle force production. These findings suggest that a supplemental ingestion of nitrate exerts beneficial effects, such as muscle performance restoration following physical activity.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

[1] Place, N., Yamada, T., Bruton, J.D. and Westerblad, H. (2010) Muscle Fatigue: From Observations in Humans to Underlying Mechanisms Studied in Intact Single Muscle Fibres. *European Journal of Applied Physiology*, **110**, 1-15. https://doi.org/10.1007/s00421-010-1480-0

[2] Bruton, J.D., Place, N., Yamada, T., Silva, J.P., Andrade, F.H., Dahlstedt, A.J., Zhang, S.J., Katz, A., Larsson, N.G. and Westerblad, H. (2008) Reactive Oxygen Species and Fatigue-Induced Prolonged Low-Frequency Force Depression in Skeletal Muscle Fibres of Rats, Mice and SOD2 Overexpressing Mice. *The Journal of Physiology*, **586**, 175-184. https://doi.org/10.1113/jphysiol.2007.147470

[3] Chin, E.R. and Allen, D.G. (1997) Effects of Reduced Muscle Glycogen Concentration on Force, Ca^{2+} Release and Contractile Protein Function in Intact Mouse Skeletal Muscle. *The Journal of Physiology*, **498**, 17-29. https://doi.org/10.1113/jphysiol.1997.sp021838

[4] Ørtenblad, N., Nielsen, J., Saltin, B. and Holmberg, H.C. (2011) Role of Glycogen Availability in Sarcoplasmic Reticulum Ca^{2+} Kinetics in Human Skeletal Muscle. *The Journal of Physiology*, **589**, 711-725. https://doi.org/10.1113/jphysiol.2010.195982

[5] Verburg, E., Dutka, T.L. and Lamb, G.D. (2006) Long-Lasting Muscle Fatigue: Partial Disruption of Excitation-Contraction Coupling by Elevated Cytosolic Ca^{2+} Concentration During Contractions. *American Journal of Physiology-Cell Physiology*, **290**, C1199-C1208. https://doi.org/10.1152/ajpcell.00469.2005

[6] Watanabe, D. and Wada, M. (2016) Predominant Cause of Prolonged Low-Frequency Force Depression Changes during Recovery after *in Situ* Fatiguing Stimulation of Rat Fast-Twitch Muscle. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **311**, R919-R929. https://doi.org/10.1152/ajpregu.00046.2016

[7] Nosaka, K. and Clarkson, P.M. (1996) Changes in Indicators of Inflammation after Eccentric Exercise of the Elbow Flexors. *Medicine and Science in Sports and Exercise*, **28**, 953-961. https://doi.org/10.1097/00005768-199608000-00003

[8] Warren, G.L., Ingalls, C.P., Lowe, D.A. and Armstrong, R.B. (2001) Excitation-Contraction Uncoupling: Major Role in Contraction-Induced Muscle Injury. *Exercise and Sport Sciences Reviews*, **29**, 82-87.

[9] Friden, J. and Lieber, R.L. (1996) Ultrastructural Evidence for Loss of Calcium Homeostasis in Exercised Skeletal Muscle. *Acta Physiologica Scandinavica*, **158**, 381-382. https://doi.org/10.1046/j.1365-201X.1996.592341000.x

[10] Proske, U. and Allen, T.J. (2005) Damage to Skeletal Muscle from Eccentric Exercise. *Exercise and Sport Sciences Reviews*, **33**, 98-104. https://doi.org/10.1097/00003677-200504000-00007

[11] Matsunaga, S., Kanzaki, K., Mishima, T., Fukuda, J., Matsunaga, S. and Wada, M. (2015) Enhanced Activity of Eccentric Contraction Induces Alterations in *In Vitro* Sarcoplasmic Reticulum Ca^{2+} Handling in Rat Hindlimb Muscles. *The Journal of Physical Fitness and Sports Medicine*, **4**, 117-124. https://doi.org/10.7600/jpfsm.4.117

[12] Cosby, K., Partovi, K.S., Crawford, J.H., Patel, R.P., Reiter, C.D., Martyr, S., Yang, B.K., Waclawiw, M.A., Zalos, G., Xu, X., Huang, K.T., Shields, H., Kim-Shapiro, D.B., Schechter, A.N., Cannon, R.O. III and Gladwin, M.T. (2003) Nitrate Reduction to Nitric Oxide by Deoxyhemoglobin Vasodilates the Human Circulation. *Nature Medicine*, **9**, 1498-1505. https://doi.org/10.1038/nm954

[13] Nisoli, E., Clementi, E., Paolucci, C., Cozzi, V., Tonello, C., Sciorati, C., Bracale, R., Valerio, A., Francolini, M., Moncada, S. and Carruba, M.O. (2003) Mitochondrial Biogenesis in Mammals: The Role of Endogenous Nitric Oxide. *Science*, **299**, 896-899. https://doi.org/10.1126/science.1079368

[14] Shiva, S. (2010) Mitochondria as Metabolizers and Targets of Nitrite. *Nitric Oxide*, **15**, 64-74. https://doi.org/10.1016/j.niox.2009.09.002

[15] Stamler, J.S. and Meissner, G. (2001) Physiology of Nitric Oxide in Skeletal Muscle. *Physiological Reviews*, **81**, 209-237. https://doi.org/10.1152/physrev.2001.81.1.209

[16] Jones, A.M. (2014) Dietary Nitrate Supplementation and Exercise Performance. *Sports Medicine*, **44**, 35-45. https://doi.org/10.1007/s40279-014-0149-y

[17] Cermak, N.M., Gibala, M.J. and van Loon, L.J. (2012) Nitrate Supplementation's Improvement of 10 km Time-Trial Performance in Trained Cyclists. *International Journal of Sport Nutrition and Exercise Metabolism*, **22**, 64-71. https://doi.org/10.1123/ijsnem.22.1.64

[18] Fulford, J., Winyard, P.G., Vanhatalo, A., Bailey, S.J., Blackwell, J.R. and Jones, A.M. (2013) Influence of Dietary Nitrate Supplementation on Human Skeletal Muscle Metabolism and Force Production during Maximum Voluntary Contractions. *Pflügers Archiv*, **465**, 517-528. https://doi.org/10.1007/s00424-013-1220-5

[19] Ivarsson, N., Schiffer, T.A., Hernández, A., Lanner, J.T., Weitzberg, E., Lundberg, J.O. and Westerblad, H. (2017) Dietary Nitrate Markedly Improves Voluntary Running in Mice. *Physiology & Behavior*, **168**, 55-61. https://doi.org/10.1016/j.physbeh.2016.10.018

[20] Hernández, A., Schiffer, T.A., Ivarsson, N., Cheng, A.J., Bruton, J.D., Lundberg, J.O., Weitzberg, E. and Westerblad, H. (2012) Dietary Nitrate Increases Tetanic $[Ca^{2+}]i$ and Contractile Force in Mouse Fast-Twitch Muscle. *The Journal of Physiology*, **590**, 3575-3583. https://doi.org/10.1113/jphysiol.2012.232777

[21] Kanzaki, K., Watanab, D., Aibara, C., Kawakami, Y., Yamada, T., Takahashi, Y. and

Wada, M. (2018) L-Arginine Ingestion Inhibits Eccentric Contraction-Induced Proteolysis and Force Deficit via S-Nitrosylation of Calpain. *Physiological Reports*, **6**. https://doi.org/10.14814/phy2.13582

[22] Ferguson, S.K., Holdsworth, C.T., Wright, J.L., Fees, A.J., Allen, J.D., Jones, A.M., Musch, T.I. and Poole, D.C. (2015) Microvascular Oxygen Pressures in Muscles Comprised of Different Fiber Types: Impact of Dietary Nitrate Supplementation. *Nitric Oxide*, **48**, 38-43. https://doi.org/10.1016/j.niox.2014.09.157

[23] Kanzaki, K., Kuratani, M., Mishima, T., Matsunaga, S., Yanaka, N., Usui, S. and Wada, M. (2010) The Effects of Eccentric Contraction on Myofibrillar Proteins in Rat Skeletal Muscle. *European Journal of Applied Physiology*, **110**, 943-952. https://doi.org/10.1007/s00421-010-1579-3

[24] Matsunaga, S., Mishima, T., Yamada, T., Inashima, S. and Wada, M. (2008) Alterations in *In Vitro* Function and Protein Oxidation of Rat Sarcoplasmic Reticulum Ca^{2+}-ATPase during Recovery from High-Intensity Exercise. *Experimental Physiology*, **93**, 426-433. https://doi.org/10.1113/expphysiol.2007.040477

[25] Bradford, M.M. (1976) A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, **72**, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

[26] Simonides, W.S. and van Hardeveld, C. (1990) An Assay for Sarcoplasmic Reticulum Ca^{2+}-ATPase Activity in Muscle Homogenate. *Analytical Biochemistry*, **191**, 321-331. https://doi.org/10.1016/0003-2697(90)90226-Y

[27] Grynkiewicz, G., Poenie, M. and Tsien, R.Y. (1985) A New Generation of Ca^{2+} Indicators with Greatly Improved Fluorescent Properties. *The Journal of Biological Chemistry*, **260**, 3440-3450.

[28] Larsen, F.J., Weitzberg, E., Lundberg, J.O. and Ekblom, B. (2007) Effects of Dietary Nitrate on Oxygen Cost during Exercise. *Acta Physiologica*, **191**, 59-66. https://doi.org/10.1111/j.1748-1716.2007.01713.x

[29] Webb, A.J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., Rashid, R., Miall, P., Deanfield, J., Benjamin, N., MacAllister, R., Hobbs, A.J. and Ahluwalia, A. (2008) Acute Blood Pressure Lowering, Vasoprotective, and Antiplatelet Properties of Dietary Nitrate via Bioconversion to Nitrite. *Hypertension*, **51**, 784-790. https://doi.org/10.1161/HYPERTENSIONAHA.107.103523

[30] Lansley, K.E., Winyard, P.G., Fulford, J., Vanhatalo, A., Bailey, S.J., Blackwell, J.R., DiMenna, F.J., Gilchrist, M., Benjamin, N. and Jones, A.M. (2011) Dietary Nitrate Supplementation Reduces the O_2 Cost of Walking and Running: A Placebo-Controlled Study. *Journal of Applied Physiology*, **110**, 591-600. https://doi.org/10.1152/japplphysiol.01070.2010

[31] Bailey, S.J., Fulford, J., Vanhatalo, A., Winyard, P.G., Blackwell, J.R., DiMenna, F.J., Wilkerson, D.P., Benjamin, N. and Jones, A.M. (2010) Dietary Nitrate Supplementation Enhances Muscle Contractile Efficiency during Knee-Extensor Exercise in Humans. *Journal of Applied Physiology*, **109**, 135-148. https://doi.org/10.1152/japplphysiol.00046.2010

[32] Haider, G. and Folland, J.P. (2014) Nitrate Supplementation Enhances the Contractile Properties of Human Skeletal Muscle. *Medicine & Science in Sports & Exercise*, **46**, 2234-2243. https://doi.org/10.1249/MSS.0000000000000351

[33] Whitfield, J., Gamu, D., Heigenhauser, G.J.F., VAN Loon, L.J.C., Spriet, L.L., Tupling, A.R. and Holloway, G.P. (2017) Beetroot Juice Increases Human Muscle Force without Changing Ca^{2+}-Handling Proteins. *Medicine & Science in Sports & Exercise*, **49**, 2016-2024. https://doi.org/10.1249/MSS.0000000000001321

[34] Lansley, K.E., Winyard, P.G., Bailey, S.J., Vanhatalo, A., Wilkerson, D.P., Blackwell, J.R., Gilchrist, M., Benjamin, N. and Jones, A.M. (2011) Acute Dietary Nitrate Supplementation Improves Cycling Time Trial Performance. *Medicine & Science in Sports & Exercise*, **43**, 1125-1131. https://doi.org/10.1249/MSS.0b013e31821597b4

[35] Wylie, L.J., Mohr, M., Krustrup, P., Jackman, S.R., Ermıdis, G., Kelly, J., Black, M.I., Bailey, S.J., Vanhatalo, A. and Jones, A.M. (2013) Dietary Nitrate Supplementation Improves Team Sport-Specific Intense Intermittent Exercise Performance. *European Journal of Applied Physiology*, **113**, 1673-1684. https://doi.org/10.1007/s00421-013-2589-8

[36] Takekura, H., Fujinami, N., Nishizawa, T., Ogasawara, H. and Kasuga, N. (2001) Eccentric Exercise-Induced Morphological Changes in the Membrane Systems Involved in Excitation-Contraction Coupling in Rat Skeletal Muscle. *The Journal of Physiology*, **533**, 571-583. https://doi.org/10.1111/j.1469-7793.2001.0571a.x

[37] Balnave, C.D., Davey, D.F. and Allen, D.G. (1997) Distribution of Sarcomere Length and Intracellular Calcium in Mouse Skeletal Muscle Following Stretch-Induced Injury. *The Journal of Physiology*, **502**, 649-659. https://doi.org/10.1111/j.1469-7793.1997.649bj.x

[38] Lavender, A.P. and Nosaka, K. (2006) Changes in Fluctuation of Isometric Force Following Eccentric and Concentric Exercise of the Elbow Flexors. *European Journal of Applied Physiology*, **96**, 235-240. https://doi.org/10.1007/s00421-005-0069-5

[39] Liao, P., Zhou, J., Ji, L.L. and Zhang, Y. (2010) Eccentric Contraction Induces Inflammatory Responses in Rat Skeletal Muscle: Role of Tumor Necrosis Factor-Alpha. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **298**, R590-R607. https://doi.org/10.1152/ajpregu.00480.2009

[40] Zhang, B.T., Whitehead, N.P., Gervasio, O.L., Reardon, T.F., Vale, M., Fatkin, D., Dietrich, A., Yeung, E.W. and Allen, D.G. (2012) Pathways of Ca^{2+} Entry and Cytoskeletal Damage Following Eccentric Contractions in Mouse Skeletal Muscle. *Journal of Applied Physiology*, **112**, 2077-2086. https://doi.org/10.1152/japplphysiol.00770.2011

[41] Ferreira, L.F. and Behnke, B.J. (2011) A Toast to Health and Performance! Beetroot Juice Lowers Blood Pressure and the O_2 Cost of Exercise. *Journal of Applied Physiology*, **110**, 585-586. https://doi.org/10.1152/japplphysiol.01457.2010

[42] Liu, R., Li, Y., Wang, M., Zhou, G. and Zhang, W. (2016) Effect of Protein S-Nitrosylation on Autolysis and Catalytic Ability of μ-Calpain. *Food Chemistry*, **213**, 470-477. https://doi.org/10.1016/j.foodchem.2016.06.104

[43] Kanzaki, K., Watanabe, D., Kuratani, M., Yamada, T., Matsunaga, S. and Wada, M. (2017) Role of Calpain in Eccentric Contraction-Induced Proteolysis of Ca^{2+}-Regulatory Proteins and Force Depression in Rat Fast-Twitch Skeletal Muscle. *Journal of Applied Physiology*, **122**, 396-405. https://doi.org/10.1152/japplphysiol.00270.2016