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Original article

Effects of caffeine ingestion on resistance exercise-induced apoptosis in athletes: A randomized, double-blind, placebo-controlled, crossover study

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Summary. Acute exercise is a stressful stimulus that may lead to systemic apoptosis. No studies to date address the apoptosis response to caffeine ingestion during acute resistance exercise (RE). The aim of this study was to determine the effects of oral caffeine ingestion on biomarkers of apoptosis including Bax and Bcl-2 during strenuous RE in resistance trained men. In a randomized, double-blind, placebo-controlled, crossover study, fourteen resistance trained men (20.6±2.3 years; 72.8±9.2 kg; 178.7±4.2 cm) ingested caffeine (6 mg/kg body weight) or placebo one hour before acute RE with 85% of one-repetition maximum (1RM). Blood samples were taken pre- (Pre), immediately post- (IP), and 15 min post- (15P) RE for measurement of serum Bax and Bcl-2 concentrations. Data were analyzed with ANOVA with repeated measures ($P<0.05$). Bax and percentage change of Bax/Bcl-2 ratio at IP RE were significantly lower in caffeine condition compared to the placebo condition. Moreover, anti-apoptotic Bcl-2 levels were significantly increased at IP in both caffeine and placebo conditions ($p=0.041$ and 0.01 respectively), but no differences were observed between both conditions at any time point ($p>0.05$). These results suggest that acute caffeine intake attenuated exercise-induced apoptosis in resistance trained men, which was confirmed by attenuated percentage change of Bax/Bcl-2 ratio in the caffeine condition.

Key words: caffeine, apoptosis, resistance exercise

Introdution

To acquire training adaptation in many athletes, progressive and intensive acute exercise is inevitable. Vigorous exercise is a form of physiological stress that promotes drastic homeostatic changes in the body which in turn induce immune dysfunction. It was previously thought that these dysfunctions were largely due to inflammatory and necrotic processes, however recent studies have illustrated that apoptosis plays an important role for in a variety of tissues (1, 2).

Apoptosis is a tightly coordinated biological process that plays a vital role in monitoring a variety of non-pathological cellular events, however deregulation of apoptosis is now recognized as a mechanism fundamental to numerous pathologies (3, 4). Vigorous exercise increases the production of reactive oxygen species (ROS) and cytokine (i.e., tumor necrosis factor-α (TNF-α), interleukin-6, etc.) levels, which can influence apoptotic signaling (2). The extrinsic and intrinsic apoptotic pathways can be involved in the apoptotic cascades. These include a cytokine/Fas receptor driven pathway, an endoplasmic reticulum–sarcoplasmic reticulum stress-induced pathway and a mitochondrial-dependent pathway (5). The mitochondrial-mediated pathway, including the Bcl-2 family, is believed to be

critical in regulating apoptosis (4, 6). Cellular stresses lead to cytochrome *c* release from the intermembrane space of mitochondria and activation of the caspase-3 (as critical effector caspase) (7, 8). Indeed, a group of proteins that initiate apoptosis (i.e., Bax, Fas, p53, etc.) and proteins that inhibit apoptosis (Bcl-2, Bcl-XL) regulate the execution of apoptosis or the outcome for the cell (death vs survival) (1). While the Bcl-2 protein regulate apoptosis by blocking the mitochondrial release of cytochrome *c*, the Bax protein act by promoting such release (9). In addition, Bcl-2 and Bax regulate Ca^{2+} homeostasis at the endoplasmic reticulum–sarcoplasmic reticulum (10). Increased Bax/Bcl-2 ratio upregulates caspase-3 and increases apoptosis in the cells (11). Few studies have investigated apoptotic markers in relation to acute eccentric exercise. Eccentric-type exercise has been shown to elevate the expression of Bcl-2, Bax, and caspase-3 staining in rat muscle fibers (12). In humans, eccentric exercise has also been shown to elevate muscle Bcl-2 and Bax protein levels (13). Since caffeine (chemically, 1,2,3-trimethylxanthine) ingestion before exercise increases endurance performance and speed and/or power output in the human studies (14, 15) have become the most popular supplement proposed as an ergogenic aid. Caffeine acts on various organs, in particular central nervous system, skeletal muscles and cardiac muscle (16, 17). Caffeine induces cells apoptosis in several cancer types (18), however, the effects of caffeine on healthy cells have not been fully elucidated. It is reported that caffeine did not vary the Bax/Bcl2 expression and so it did not induce apoptosis (19). In another trial, caffeine reduces the expression level of Bcl-2 and does not affect expression of Bax (18). Hence, caffeine may influence tissue function modifying the cellular life-death cycle.

Due to the possible effects of caffeine on ROS (17), Ca^{2+} homeostasis (20) and cytokines (21), it may affect cells apoptosis during intense exercise. No study to date has addressed the effect of caffeine supplementation on exercise-induced apoptosis. Therefore, the aim of this study was to examine the effect of caffeine on apoptosis biomarkers including Bax (pro-apoptotic protein) and Bcl-2 (anti-apoptotic protein) after intense RE in athletes.

Methods

Subjects

Fourteen resistance-trained men voluntarily participated in a randomized, double-blind, placebo-controlled, crossover study which was approved by the Institutional Review Board of the University. This study was limited to males because of gender differences in the response of apoptosis markers to eccentric exercise (13). All subjects were low users of caffeine (≤50 $mg.d^{-1}$). The general characteristics of the subjects are presented in Table 1. All the participants were resistance trained (resistance training for at least 2 year). Exclusionary criteria included use of androgens or nutritional supplements within the previous 3 months, taking any type of medication or smoking, neurological or chronic inflammatory disease, cardiac arrhythmia interfering with physical function, peripheral vascular disease and any absolute contraindications to acute exercise. All subjects were informed of the purpose, procedures and possible risks of the investigation before they gave written informed consent to participate in the study.

Procedures

Subjects participated in a randomized, double-blind, placebo-controlled, crossover study to evaluate the influence of caffeine ingestion on serum biomarkers of apoptosis during acute RE. Subjects were asked to attend three laboratory sessions which includes one familiarization session and two testing sessions. At least 7 days prior to the experiments, after anthropometric data collection and 1 repetition maximum (1RM) strength measurements, the participants were

Table 1. Subjects physical characteristics

Variable	***mean ± SD***	
	Caffeine	Placebo
Age (y)	20.6±2.3	20.6±2.4
Weight (kg)	72.9±9.2	72.8±9.3
Height (cm)	178.7±4.2	178.7±4.3
BMI (kg/m^2)	22.8±2.4	22.8±2.6
BMR (kcal/day)	2035.8±184	2049.1±193
Body fat (%)	12.1±4.1	12.1±4.3
Body fat (kg)	9.0±3.8	9.0±3.9
Fat-free mass (kg)	60.4±6.2	60.4±6.4

familiarized with the exercise scheme during study orientation. The 1RM test was perfected for each of the four RE and 85% of 1RM selected as represented workload used in the experimental sessions. The subjects then completed three sets to failure repetitions of four different RE at 85% of 1RM as a familiarization session to acute exercise session. The order of RE was as follows: supine bench press, shoulder press, lat pull-down and leg press with an interval of 2 min between sets and the exercises.

The following 2 sessions, upon arrival to the laboratory, subjects consumed gelatin capsules containing 6 mg.kg^{-1} body mass of caffeine (CA, C0750 Sigma-Aldrich, Germany) or Maltodextrin as placebo. After 60 minutes (22), subjects performed acute RE protocol with a short warm-up. The two trial periods were separated by a 5 day wash-out period (14). Participants were directed to avoid any intense exercise and from consumption of caffeinated foods and beverages for 72 hours before the exercise sessions. Body composition was determined with a Body Composition Analyzer (InBody 3.0, Biospace, Seoul, Korea)

During the exercise sessions, blood samples (6 mL) were obtained via venipuncture at pre-exercise (Pre), immediately post (IP), and 15 min post (15P) RE for measurement of serum concentrations Bax and Bcl-2 proteins. The tubes were kept in the dark and refrigerated on ice until the end of the test. The blood was processed and centrifuged at 3,000 revolutions per minute for 20 minutes. The Bax and Bcl-2 serum levels were determined using commercial kits by sandwich ELISA (Human ELISA, Bioassay Technology laboratory, China) according to the manufacturer's protocol. The serum Bax sensitivity was 0.15 ng/ml and the inter- and intra-assay variability were <10% and <8%, respectively (23). The serum Bcl-2 sensitivity was 1.15 U/ml and the inter- and intra-assay variability were <10% and <8%, respectively (23).

Statistical analyses

All data are expressed as means ± SD. The Shapiro–Wilk test of the normality of distribution was performed for all variables before the analysis. The homogeneity of variance was calculated by the Levene test. Within-group (from pre- to post- exercise) and between-group (caffeine vs. placebo) comparisons were carried out using an analyze variance (ANOVA) with repeated measure and post-hoc Bonferroni test. A significance level of $p<0.05$ was accepted (3 time × 2 group) as statistically significant. Data analysis was undertaken using the IBM SPSS software for Windows, version 22.0.

Results

There were no significant baseline differences between caffeine and placebo conditions in Bax (p=0.26) and Bcl-2 concentration (p=0.27). The effect of caffeine ingestion compared with placebo on serum Bax concentrations are shown in Figure 1. Serum Bax concentration was significantly lower in the caffeine ingestion than in the placebo ingestion at IP (p=0.004) and 15P RE (p=0.04). Significant increase in Bax concentration was seen in placebo condition at IP (p=0.001) but returned to Pre concentrations by 15P (p=0.38). Serum Bax concentrations were observed to decrease from Pre at IP (p=0.019) and 15P (p=0.012) in caffeine condition. Changes in Bcl-2 concentrations can be observed in Figure 2. Increases from Pre were seen in caffeine and placebo conditions at IP (p=0.041 and 0.01 respectively), but no differences were observed between both conditions at IP (p=0.19) and 15P (p=0.34). In addition, the percentage change of Bax/Bcl-2 ratio was significantly lower in caffeine condition than in placebo at IP (p=0.01) and 15P (p=0.032).

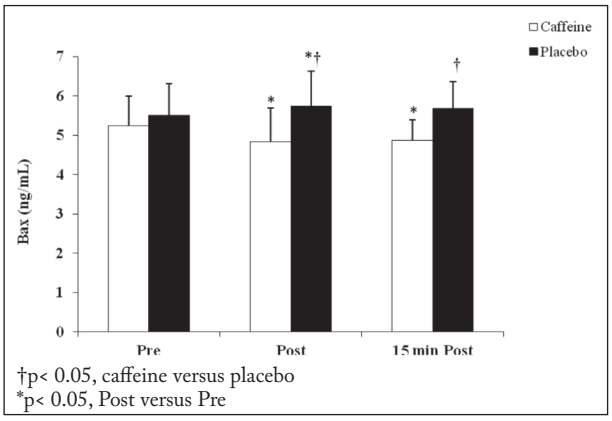

Figure 1. Serum level responses of Bax to a bout of resistance exercise after 30 min of caffeine or placebo supplementation.

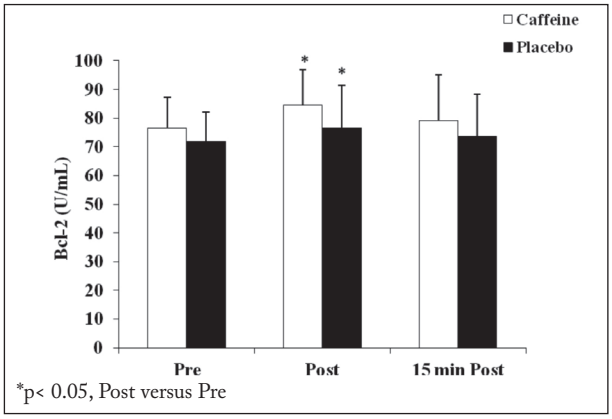

Figure 2. Serum level responses of Bcl-2 to a bout of resistance exercise after 30 min of caffeine or placebo supplementation.

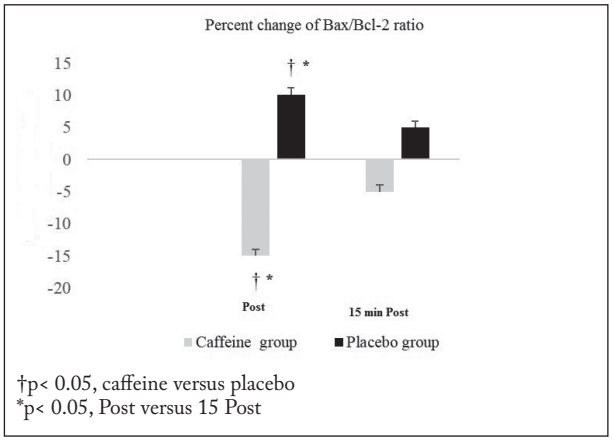

Figure 3. percentage change of Bax/Bcl-2 ratio to a bout of resistance exercise after 30 min of caffeine or placebo supplementation.

Discussion

Caffeine is now appearing in many new products, including energy drinks and sport gels. Moderate consumption of caffeine may decrease the risk of a number of muscular, neurological and cardiovascular diseases (such as Dementia/Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease) in recent decades (24). Some studies have suggested that caffeine could be an effective factor against exercise-induced ROS (25) and inflammation (26). However, the role of caffeine supplementation in exercise-induced apoptosis remains unclear. Indeed, Bax and Bcl-2 levels are critical intracellular checkpoint of apoptosis within a distal common cell death pathway (19). The goal of the current study, therefore, was to examine the impact of caffeine in healthy athletes exposed to acute RE on serum Bax and Bcl-2 levels. For this purpose, Bax and Bcl-2 proteins as important apoptotic regulators of apoptosis were measured in response to caffeine ingestion during intense RE in a randomized, double-blind, placebo-controlled, crossover study.

The main findings of the present study were that caffeine ingestion 1h prior to RE inhibited the increase in pro-apoptotic Bax protein and the percentage change of Bax/Bcl-2 ratio induced by RE in resistance trained men. Furthermore, intense RE resulted to a marked raise in pro-apoptotic Bax protein and the ratio of Bax/Bcl-2 in resistance trained men. Corroborating to these findings, it was previously demonstrated increases in pro-apoptotic proteins following strenuous exercise in humans (27-29) and animals (30, 31). In this respect, Parke et al. (29) reported a significant increases in Bax concentration and the Bax/Bcl-2 ratio at 24 and 48h post 40 min downhill running in moderately trained subjects. Moreover, other investigation observed an increase in the muscle Bax protein and Bax/Bcl-2 ratio at 6h after performing 7 sets of 10 eccentric repetitions of the knee extensors at 150% of 1RM in recreationally active men (13). Also, recently significant increases in muscle levels of Bax and Bcl-2 were observed after one set of (100 repetitions at 30°/s) eccentric exercise of the dominant knee extensors in health active males.

Animal studies regarding to pro-/anti-apoptotic proteins response to exercise show different findings (30-32). Quadrilatero and Hoffman-Goetz (31) reported a significant decrease in anti-apoptotic Bcl-2 protein level and an increase in pro-apoptosis caspase-3 protein level after strenuous exercise in intestinal lymphocyte of mice. Also, Lagranha et al. (30) reported that strenuous exercise resulted significant increases in the expression of the pro-apoptotic genes Bax and Bcl-x_s and decreases in the expression of the anti-apoptotic gene Bcl-x_L in mice neutrophils following a 1 h wheel-running exercise. However, Podhorska-Okolow et al. (32) observed a reduction in mouse muscle Bcl-2 level with no change in Bax and Fas concentration after 16 h spontaneous wheel exercise.

Levels of Bcl-2 protein were also measured as important anti-apoptotic protein after RE. Bcl-2 protein

can inhibit the mitochondrial apoptotic pathway via binding to its pro-apoptotic protein Bax and permeability transition pore, thereby preventing the release of cytochrome *c* and activation of the apoptotic cascade (10). Bcl-2 protein level in the present study increases in response to storehouse RE in resistance trained men. Corroborating to these findings, Kerksick et al. (33) found significant increases in muscle levels of Bcl-2 after eccentric exercise in healthy active men. In another study by Kerksick et al.(13) eccentric exercise induced significant increase in muscle Bcl-2 levels in women but not in men. In contrast, Park et al. (34) found no change in Bcl-2 immediately, 2h, 24h, and 48h after acute eccentric exercise in trained subjects. Also, no changes in Bcl-2 were found after intense RE in healthy men (23).

Possible mechanisms underlying the resistance exercise-induced apoptosis have not been fully clarified, but it is apparent from the previous studies that intense exercise can cause muscle damage, as well as provoke increases in ROS, DNA damage, and concentrations of pro-inflammatory cytokines (1, 2, 29). All of these ways can modulate exercise-induced apoptosis process. In this regard, previous studies have shown that DNA damage triggers signal cascades in the cytoplasm, which ultimately leads to the phosphorylation of p53, which, in turn, enhances pro-apoptotic protein Bax concentration and reduces anti-apoptotic protein Bcl-2 concentration (30, 32). Bax is transcriptional target for the p53, which promotes apoptosis in response to DNA damage, whereas, protein Bcl-2 protects cells against oxidative stress and apoptosis (35, 36). The role of Bax in promoting apoptosis attributed to the release of cytochrome *c* into the cytosol which actively participate in the apoptosis process (7).

To date, there are no studies to evaluate the effect of caffeine ingestion on pro-/anti-apoptotic proteins following intense RE in athletes. To our knowledge, this is the first study to show acute caffeine ingestion inhibited RE-induced increase in pro-apoptotic Bax protein and percentage change of Bax/Bcl-2 ratio. Previous studies using different forms of antioxidants have reported a beneficial effect of N-acetyl-l-cysteine (NAC) (31), ascorbic acid (37), catalas (38) on lymphocytes apoptosis.

Based on previous study, it seems that attenuated levels of Bax may be partially caused by suppression of p53 induced by caffeine ingestion (39). Moreover, most studies have found a preventive effect of caffeine on ROS during acute exercise (17, 25). Combined, these results suggest that caffeine may attenuate apoptosis by inhibiting Bax in acute eccentric exercise. On the other hand, the physiological studies reported that caffeine independently inhibits the proliferation, and induces apoptosis of tumour cells (40). Additionally, caffeine could induce a dose-dependent apoptosis in Bax-overexpressing normal cells (41). Caffeine may stimulate the muscular apoptosis dose-dependently: low-dose caffeine intake prevents apoptosis, whereas high-dose caffeine intake stimulates it (42). Although we did not measure the different caffeine doses and cancers cells, our novel findings reveal that low caffeine does suppress Bax in normal tissue cells after acute exercise.

In addition, RE-induced increase in Bax/Bcl-2 ratio was prevented by the ingestion of 6 mg of caffeine per kilogram of body. The ratio of Bax/Bcl-2 determine the susceptibility of a cell to apoptosis and these ratio are a reliable index for apoptosis (43). Our findings of elevated Bax/Bcl-2 ratio following acute RE support previous research on healthy men (23). Caffeine ingestion leads to significant decrease in percent of Bax/Bcl-2 ratio after acute RE. Based on these findings it could be concluded that caffeine appears to attenuate susceptibility of cells to apoptosis, whereas RE alone could accelerate apoptosis.

Our findings also indicate that caffeine ingestion did not affect anti-apoptotic protein Bcl-2 level after strenuous RE. There are very little data regarding the Bcl-2 after acute caffeine consumption. Some studies have reported that acute caffeine administration caused a transitory decrease of anti-apoptotic Bcl-2 expression in the skeletal muscle (18, 44). However, Corsetti et al. (2008) observed that caffeine treatment did not affect Bcl-2 in the heart (19). These data demonstrated that possible effect of caffeine on Bcl-2 may most likely be dependent on cell type (45). However, we assessed serum Bcl-2 (as systemic anti-apoptotic protein), which may be released from any cell types. It will be of interest to do additional studies on different tissues after caffeine intake in combination with acute RE.

The present study possesses some relevant limitations. As previous studies (27, 46), major limitation is that only serum Bax and Bcl-2 markers of apoptosis

(which may be released from any cell types) in relation to RE were assessed. Thus, further research should elucidate the protective systemic and/or local effects of caffeine on different pro/anti-apoptotic factors in different types of tissue exposed to RE. Second, we studied the impact of 6 mg/kg caffeine on Bax and Bcl-2 markers concentrations. It is known that caffeine doses might influence apoptosis responses in certain situations (42, 47). Therefore, it is recommended that future studies address the effect of varying dose of caffeine on Bax and Bcl-2 proteins following intense RE in athletes.

To our knowledge, this is the first study to investigate Bax and Bcl-2 response to caffeine intake following RE in resistance trained men. In summary, the data from the present study show that the ingestion of 6 $mg.k^{-1}$.BW caffeine 1h before the onset of intense RE significantly decreased exercise-induced apoptosis. Caffeine ingestion was associated with decreased Bax concentration and Bax/Bcl-2 ratio immediately after RE with 85% of 1RM, yet there were no significant differences observed for anti-apoptotic Bcl-2 responses following RE in resistance trained men.

This caffeine effect could be related to the modulation of mitochondrial ROS production and p53. It can be speculated that caffeine-mediated inhibition of Bax may contribute to prevent cells apoptosis in strenuous RE. Our data provide the experimental basis for further studies of potential role of caffeine on the exercise-induced apoptosis.

References

1. S. Phaneuf, C. Leeuwenburgh, Apoptosis and exercise, Medicine and Science in Sports and Exercise 33(3) (2001) 393-396.
2. J. Quadrilatero, S.E. Alway, E.E. Dupont-Versteegden, Skeletal muscle apoptotic response to physical activity: potential mechanisms for protection, Applied Physiology, Nutrition, and Metabolism 36(5) (2011) 608-617.
3. H.-B. Kwak, Effects of aging and exercise training on apoptosis in the heart, Journal of Rehabilitation 9(2) (2013) 212-219.
4. A. Jafari, H. Pourrazi, S. Nikookheslat, B. Baradaran, Effect of Exercise Training on Bcl-2 and Bax Gene Expression in the Rat Heart, Gene, Cell and Tissue 2(4) (2015).
5. E. Marzetti, G. Privitera, V. Simili, S.E. Wohlgemuth, L. Aulisa, M. Pahor, C. Leeuwenburgh, Multiple pathways to the same end: mechanisms of myonuclear apoptosis in sarcopenia of aging, The Scientific World Journal 10 (2010) 340-349.
6. J. Marín-García, M.J. Goldenthal, Mitochondrial centrality in heart failure, Heart Failure Reviews 13(2) (2008) 137-150.
7. V. Gogvadze, S. Orrenius, B. Zhivotovsky, Multiple pathways of cytochrome c release from mitochondria in apoptosis, Biochimica Et Biophysica Acta (BBA)-Bioenergetics 1757(5) (2006) 639-647.
8. D.R. Mcilwain, T. Berger, T.W. Mak, Caspase functions in cell death and disease, Cold Spring Harbor Perspectives in Biology 5(4) (2013) a008656.
9. C. Wang, R.J. Youle, The role of mitochondria in apoptosis, Annual Review of Genetics 43 (2009) 95.
10. Y. Rong, C.W. Distelhorst, Bcl-2 protein family members: versatile regulators of calcium signaling in cell survival and apoptosis, Annual Review of Physiology 70 (2008) 73-91.
11. E. Bagci, Y. Vodovotz, T. Billiar, G. Ermentrout, I. Bahar, Bistability in apoptosis: roles of bax, bcl-2, and mitochondrial permeability transition pores, Biophysical Journal 90(5) (2006) 1546-1559.
12. M. Sudo, Y. Kano, Myofiber apoptosis occurs in the inflammation and regeneration phase following eccentric contractions in rats, The Journal of Physiological Sciences 59(6) (2009) 405-412.
13. C. Kerksick, L. Taylor, A. Harvey, D. Willoughby, Gender-related differences in muscle injury, oxidative stress, and apoptosis, Medicine and Science in Sports and Exercise 40(10) (2008) 1772.
14. T.E. Graham, Caffeine and exercise, Sports Medicine 31(11) (2001) 785-807.
15. C.J. Hulston, A.E. Jeukendrup, Substrate metabolism and exercise performance with caffeine and carbohydrate intake, Medicine and Science in Sports and Exercise 40(12) (2008) 2096-2104.
16. J.M. Kalmar, The influence of caffeine on voluntary muscle activation, Medicine and Science in Sports and Exercise 37(12) (2005) 2113-2119.
17. S. Yenisetti, Beneficial Role of Coffee and Caffeine in Neurodegenerative Diseases: A Minireview, (2016).
18. J. Jiang, Y. Lan, T. Zhang, M. Yu, X. Liu, L. Li, X. Chen, The in vitro effects of caffeine on viability, cycle cycle profiles, proliferation, and apoptosis of glioblastomas, European Review for Medical and Pharmacological Sciences 19(17) (2015) 3201-7.
19. G. Corsetti, E. Pasini, D. Assanelli, R. Bianchi, Effects of acute caffeine administration on NOS and Bax/Bcl2 expression in the myocardium of rat, Pharmacological Research 57(1) (2008) 19-25.
20. S. Cappelletti, P. Daria, G. Sani, M. Aromatario, Caffeine: cognitive and physical performance enhancer or psychoactive drug?, Current Neuropharmacology 13(1) (2015) 71-88.
21. J.L. Cechella, M.R. Leite, F. Dobrachinski, J.T. Da Rocha, N.R. Carvalho, M.M. Duarte, F.A. Soares, G. Bresciani, L.F. Royes, G. Zeni, Moderate swimming exercise and caffeine supplementation reduce the levels of inflammatory cytokines without causing oxidative stress in tissues of middle-aged rats, Amino Acids 46(5) (2014) 1187-1195.
22. S. Teekachunhatean, N. Tosri, N. Rojanasthien, S. Srichairatanakool, C. Sangdee, Pharmacokinetics of caffeine following a single administration of coffee enema versus oral coffee con-

sumption in healthy male subjects, ISRN Fharmacology 2013 (2013).
23. V. Sari-Sarraf, R. Amirsasan, D. Sheikholeslami-Vatani, H. Faraji, Effect of creatine supplementation on the factors involved in apoptosis-related process (Bax, Bcl-2) and their ratio (Bcl-2/Bax) during acute resistance exercise in middle-aged men, Scientific Journal of Kurdistan University of Medical Sciences 21(4) (2016).
24. D.-C. You, Y.-S. Kim, A.-W. Ha, Y.-N. Lee, S.-M. Kim, C.-H. Kim, S.-H. Lee, D. Choi, J.-M. Lee, Possible health effects of caffeinated coffee consumption on Alzheimer's disease and cardiovascular disease, Toxicological Research 27(1) (2011) 7.
25. R.P. Barcelos, M.A. Souza, G.P. Amaral, S.T. Stefanello, G. Bresciani, M.R. Fighera, F.A.A. Soares, N.V. Barbosa, Caffeine supplementation modulates oxidative stress markers in the liver of trained rats, Life Sciences 96(1) (2014) 40-45.
26. R.P. Barcelos, M.A. Souza, G.P. Amaral, S.T. Stefanello, G. Bresciani, M.R. Fighera, F.A.A. Soares, N. de Vargas Barbosa, Caffeine intake may modulate inflammation markers in trained rats, Nutrients 6(4) (2014) 1678-1690.
27. S. Boroujerdi, R. Rahimi, The apoptotic response to resistance exercise with different intensities in athletes, Medicina dello Sport 64(1) (2011) 31-44.
28. H. Sharafi, R. Rahimi, The effect of resistance exercise on p53, caspase-9, and caspase-3 in trained and untrained men, The Journal of Strength & Conditioning Research 26(4) (2012) 1142-1148.
29. K.-S. Park, D.A. Sedlock, J.W. Navalta, M.-G. Lee, S.-H. Kim, Leukocyte apoptosis and pro-/anti-apoptotic proteins following downhill running, European Journal of Applied Physiology 111(9) (2011) 2349-2357.
30. C.J. Lagranha, S.M. Senna, T.M. de Lima, E. Silva, R. Curi, T. Pithon-Curi, Beneficial effect of glutamine on exercise-induced apoptosis of rat neutrophils, Medicine and Science in Sports and Exercise 36(2) (2004) 210-217.
31. J. Quadrilatero, L. Hoffman-Goetz, N-acetyl-l-cysteine protects intestinal lymphocytes from apoptotic death after acute exercise in adrenalectomized mice, American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 288(6) (2005) R1664-R1672.
32. M. Podhorska-Okolow, M. Sandri, S. Zampieri, B. Brun, K. Rossini, U. Carraro, Apoptosis of myofibres and satellite cells: exercise-induced damage in skeletal muscle of the mouse, Neuropathology and Applied Neurobiology 24(6) (1998) 518-531.
33. C.M. Kerksick, R.B. Kreider, D.S. Willoughby, Intramuscular adaptations to eccentric exercise and antioxidant supplementation, Amino Acids 39(1) (2010) 219-232.
34. K.-S. Park, D.A. Sedlock, J.W. Navalta, Exercise-Induced Muscle Damage and Apoptotic Protein Expression in Immune Cells, The FASEB Journal 21(6) (2007) A1345.
35. K.D. McCullough, J.L. Martindale, L.-O. Klotz, T.-Y. Aw, N.J. Holbrook, Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state, Molecular and Cellular Biology 21(4) (2001) 1249-1259.
36. N. Mirkovic, D.W. Voehringer, M.D. Story, D.J. McConkey, T.J. McDonnell, R.E. Meyn, Resistance to radiation-induced apoptosis in Bcl-2-expressing cells is reversed by depleting cellular thiols, Oncogene 15(12) (1997) 1461-1470.
37. J.D. Campbell, M. Cole, B. Bunditrutavorn, A.T. Vella, Ascorbic acid is a potent inhibitor of various forms of T cell apoptosis, Cellular Immunology 194(1) (1999) 1-5.
38. J. Torres-Roca, H. Lecoeur, C. Amatore, M. Gougeon, The early intracellular production of a reactive oxygen intermediate mediates apoptosis in dexamethasone-treated thymocytes, Cell Death and Differentiation 2(4) (1995) 309-319.
39. A. Tichý, D. Muthná, J. Vávrová, J. Pejchal, Z. Šinkorová, L. Zárybnická, M. Řezáčová, Caffeine-suppressed ATM pathway leads to decreased p53 phosphorylation and increased programmed cell death in gamma-irradiated leukaemic molt-4 cells, Journal of Applied Biomedicine 9(1) (2011) 49-56.
40. S. Miwa, N. Sugimoto, N. Yamamoto, T. Shirai, H. Nishida, K. Hayashi, H. Kimura, A. Takeuchi, K. Igarashi, A. Yachie, Caffeine induces apoptosis of osteosarcoma cells by inhibiting AKT/mTOR/S6K, NF-κB and MAPK pathways, Anticancer Research 32(9) (2012) 3643-3649.
41. L. Dubrez, J.-L. Coll, A. Hurbin, E. Solary, M.-C. Favrot, Caffeine sensitizes human H358 cell line to p53-mediated apoptosis by inducing mitochondrial translocation and conformational change of BAX protein, Journal of Biological Chemistry 276(42) (2001) 38980-38987.
42. M. Jafari, A. Rabbani, Studies on the mechanism of caffeine action in alveolar macrophages: caffeine elevates cyclic adenosine monophosphate level and prostaglandin synthesis, Metabolism 53(6) (2004) 687-692.
43. P.E. Czabotar, G. Lessene, A. Strasser, J.M. Adams, Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy, Nature Reviews Molecular Cell Biology 15(1) (2014) 49-63.
44. G. Corsetti, E. Pasini, D. Assanelli, E. Saligari, M. Adobati, R. Bianchi, Acute caffeine administration decreased NOS and Bcl2 expression in rat skeletal muscles, Pharmacological Research 55(2) (2007) 96-103.
45. Z. He, W.-Y. Ma, T. Hashimoto, A.M. Bode, C.S. Yang, Z. Dong, Induction of apoptosis by caffeine is mediated by the p53, Bax, and caspase 3 pathways, Cancer Research 63(15) (2003) 4396-4401.
46. H. Faraji, Rahimi, R., Sheikholeslami, V.D., Jafaari, A., Apoptosis response to different rest periods after resistance exercise in athletes, Medicina dello Sport 2016; 69(2): 173-83.
47. M. Fernandez, A. Lopez, A. Santa-Maria, Apoptosis induced by different doses of caffeine on Chinese hamster ovary cells, Journal of Applied Toxicology 23(4) (2003) 221-224.

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