Effects of estrogen on gastrocnemius muscle strain injury and regeneration in female rats[1]

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KEY WORDS estrogen; skeletal muscle; muscle injury; antioxidant; regeneration; desmin

ABSTRACT

AIM: To study the effects of estrogen on muscle damage and regeneration after acute passive gastrocnemius muscle strain injury in female Sprague-Dawley rats. **METHODS:** Rats were divided into 5 groups: ovariectomized, strained and treated with low-dosage estradiol (20 μg/d) (E_{low}), treated with high-dosage estradiol (200 μg/d) (E_{high}), treated with oil placebo (Oil), strained with no ovariectomy (Strain), and sham operated with no strain and no ovariectomy (Con). Muscle damage index [plasma creatine kinase (CK)], antioxidant indexes [glutathione (GSH), Vitamin E (Vit E), total antioxidant capability (TAC)], and muscle regeneration index (desmin) were investigated at 7 d. **RESULTS:** The plasma CK activity increased but GSH, Vit E, and TAC levels decreased after muscle strain injury (Strain *vs* Con $P<0.05$). Plasma CK activity was the greatest while GSH, Vit E, and TAC were the lowest in the Oil group among the five groups ($P<0.01$). Plasma CK in the E_{high} and Strain groups was lower than that in the E_{low} group. Plasma GSH, Vit E, and TAC were higher in the E_{high} and Strain groups compared with the E_{low} group ($P<0.05$). The expression of desmin in the E_{high} and Strain groups was higher than that in the E_{low} group ($P<0.01$) while that in the Oil group was the lowest in all the five groups ($P<0.01$). **CONCLUSION:** Endogenous estrogen in normal female rats or exogenous estrogen in ovariectomized rats could improve antioxidant capability *in vivo*, so that reduced muscle damage and accelerated muscle regeneration post gastronemius muscle strain injury.

INTRODUCTION

Muscle strain injury is one of the most frequent occurrences in sports medicine including professional and recreational sports injuries and is a challenging problem in traumatology. It has been reported that the recovery of injured muscle depends on the extent and scope of injured tissues including muscles, blood vessls, nerve fibers, the following inflammation, ischemia, oxygen deficiency, peroxidation induced by production of free radicals[1-5] and other factors such as the age, sex[6], fatigue condition[7] at that time, *etc*. Within all the factors, the role of sex hormones is increasingly drawing more attentions. For instance, there are reports of gender-based differences in exercise-induced muscle injury in humans and animal models[8,9]. Plasma CK activity[10,11], inflammatory response, leukocyte infiltration[12], and ultrastructural disruptions[9] in female have been reported lower than those in male animals. It has also been reported that estrogen complementation could attenuate neutrophil infiltration and indexes of muscle damage

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(CK, MPO) following ischemia/reperfusion (I/R) in ovariectomized rats[13] and post-exercise skeletal muscle in male and ovariectomized female rats[14,15]. All these have been attributed to the female sex steroid hormone 17β-estradiol, which may act as an antioxidant or membrane stabilizer[16-19]. Furthermore, estrogen could maintain Vit E[20, 21] and increase GSH (two important and powerful antioxidants)[22] levels when they were consumed by the increase of oxygen free radicals in body post-exercises and I/R muscle damage, which suggested that estrogen might offer an additional line of defence against oxygen free radicals and may render skeletal muscle less susceptible to oxidative damage in muscle injury induced by exercises[23]. However, the roles that estrogen plays in muscle damage and regeneration in acute passive strain injured muscles are still not clear. In this study we investigated the effects of estrogen on muscle damage (CK activity) and antioxidant indexes (Vit E, GSH and TAC) after acute passive muscle strain injury in female rats with or without ovariectomy. At the same time we observed the effect of estrogen on muscle regeneration by detecting the expression of desmin (muscle skeleton protein), which has been found to uniformly express in the regenerating myofibers and whose expression level was used as an indicator of muscle regeneration[24,25].

MATERIALS AND METHODS

Reagents Estradiol benzoate sesame oil, creatin kinase kit, estradiol immunoassay kit, and rabbit anti-desmin antibody were purchased from Sigma. Goat anti-rabbit antibody was obtained from New England Biolabs. The other reagents were of analytical purity.

Experimental instruments Instron Model TM-M materials testing system (TE-10, Beihang University, Beijing, China), spectrophotometer (Beckman DU-7400, Beckman Instruments, Fullerton, CA), HPLC pump (Waters 510, Milipore, corp), image analysis system (LEICA Q550CM, LEICA German).

Animals Forty-eight female Sprague-Dawley rats (240-260 g) were housed with controlled temperature 21±2 ºC and of 12:12-h light-dark cycle. All animals were free to access food and water. All procedures regarding the use and handling of animals were reviewed and approved by Peking University Health Science Center Animal Care and Use Committee.

Preparation of animal models Female rats were anesthetized (Chloral Hydrate, 400 mg/kg, ip) and ovariectomized via bilateral dorsal incisions[26]. Fourteen days after ovariectomy the controlled strain injury of the gastrocnemius muscle was produced on the right hindlimb of the experimental rats as described by Nikolaou PK and Almeskinders LC[4,27]. In brief, the right hindlimb distal tendon of each gastrocnemius muscle was exposed and a needle was inserted transversely through the proximal tibia, and fixed on a materials testing system. The distal tendon was connected to the load cell by the surgical silk and the muscle was pulled at a velocity of 6 cm/min. Same operation was performed on the Con group rats without strain.

Controlling of strain injury severity To simulate a clinical setting of acute passive muscle strain injury, we stopped the stretch as soon as the deformation curve reached the horizontal plateau, where the majority of injuries to muscles are partial tears and not total ruptures. This controlled stretch could keep roughly the same injury severity in all the rats as reported by Obremsky *et al*[28].

Intervention on animal models Once the rats were strained, different interventions were performed onto the rats daily for 6 d: E_{low} (n=10, 20 μg EB in 100 μL/d, estradiol benzoate sesame oil, sc), E_{high} (n=10, 200 μg BE in 100 μL/d, sc), and Oil (n=10, 100 μL sesame oil·d^{-1}, sc). On d 7, all the rats were anesthetized. Blood from the descending aorta was centrifuged immediately and plasma fraction was frozen at -80 ºC until analysis for CK, GSH, Vit E, TAC, and estradiol. The gastronemius muscles were isolated, soaked in the 4 % formaldehyde PBS solution (pH=7), paraffin embedded, and sliced into sections (6 μm). The expression of desmin was detected with immunohistochemistry.

Radioimmunoassay for plasma estradiol concentration Plasma estradiol was determined by radioimmunoassay using a double-antibody estradiol procedure according to the manufacturer's instructions.

Assay for concentrations of plasma CK activity, GSH, Vit E, and TAC Plasma total CK level was measured spectrophotometrically at 340 nm using commercially available kits. Glutathione was determined using a standard enzymatic recycling procedure as described by Tietz and modified by Anderson[29,30]. Vit E was measured with a standard high performance liquid chromatography (HPLC) technique described by Lee *et al*[31]. Total antioxidant capacity (TAC) was determined using reagents, equipment and procedures described by Whitehead *et al* [31].

Detection of desmin by immunohistochemistry Briefly, 6-μm paraffin sections were obtained from

three random gastrocnemius muscles of each group. The expression of desmin was monitored by immunohistochemistry using rabbit antidesmin antibody (1/100) and goat antirabbit antibody (1/200)[24,25]. The integra optical density of desmin positive fields was observed under microscope and computerized as index of muscle regeneration. Briefly, 10-2 random fields (similar size area 331647.57 μm^2) were selected for each section, then the desmin positive area and mean absorbance of each image field were detected. Integra absorbance =positive area×mean absorbance.

Statistical analysis All data were presented as mean±SD and processed with the SPSS 11.0 statistical software package. All statistical comparisons were made using one-way ANOVA tables with 5 groups. The Dunnett *t*-test was used for two-sided statistical analysis. The level of statistical significance was set at $P<0.05$.

RESULTS

Plasma estradiol concentration The Strain group had a similar plasma estradiol concentration compared with the Con group ($P=0.39$). Plasma estradiol contents in the Oil or E_{low} group was significantly lower than that in the Con ($P<0.01$), while the E_{high} group had a higher estradiol content compared with the Con ($P<0.01$). Plasma estradiol content in the Oil group was lower than that in the E_{high} or E_{low} group ($P<0.01$) and estradiol in the E_{high} group was higher than that in the E_{low} group ($P<0.01$) (Tab 1).

Plasma CK activity after muscle strain injury Plasma CK activity was higher in that in the Strain rats (179.0±11.7 U/L) than the Con rats (101.8±11.6 U/L) ($P<0.05$) on 7 d post injury. CK activity in the Oil group was the highest among all the groups, and that in the E_{high} group was lower compared with the E_{low} ($P < 0.05$) but similar with the Strain ($P>0.05$) (Tab 1).

Plasma Vit E, GSH, and TAC levels post muscle strain injury Plasma Vit E, GSH, and TAC decreased after muscle strain injury ($P<0.05$, *vs* Con). The Oil group had the lowest levels of Vit E, GSH, and TAC among all the groups. Plasma Vit E, GSH, and TAC in the E_{high} or Strain group were higher than those in the E_{low} group ($P<0.05$). Plasma Vit E, GSH, and TAC concentrations were similar between the E_{high} group and the Strain group ($P>0.05$) (Tab 1).

Expression of desmin post muscle strain injury Desmin expression was detected with immunohistochemistry (Fig 1) and quantified with IOD of desmin positive area. IOD in the E_{low}, E_{high} and Strain group was higher than that in the Oil group, respectively ($P<0.01$). The E_{high} or Strain group had higher IOD compared with the E_{low} ($P<0.05$). There was no significant difference of IOD between the E_{high} and the Strain group ($P>0.05$) (Fig 2).

DISCUSSION

Endogenous and exogenous estradiol administration in ovariectomized rats could protect the muscle from the sports-related chronic myofiber injury[9,11]. We found that estrogen could not only protect the muscle from acute passive strain injury but also improve regeneration of strain injured muscles in female rats. These effects may be achieved by the membrane stabilizing and antioxidant functions of estrogen[11].

Estradiol is the most important type of estrogen in the body. Estradiol benzoate is an ester of estradiol, which could transform into estradiol when injected subcutaneously in oil solution. In our study, two weeks after the ovariectomy, the endogenous estradiol in female rats were exhausted completely and exogenous benzoate estradiol administration increased the plasma estradiol concentration dose-dependently in the ovariec-

Tab 1. Plasma concentrations of estradiol, CK, Vit E, GSH, and TAC in the 5 groups after 6 d of muscle strain injury. Means±SD. $^bP<0.05$, $^cP < 0.01$ *vs* Con. $^eP<0.05$, $^fP<0.01$ *vs* Oil.

		Plasma estradiol/ng·L^{-1}	CK/U·L^{-1}	Vit E/mg·L^{-1}	GSH/mmol·L^{-1}	TAC/μmol Trolox Eq·L^{-1}
Con	(n=8)	34.0±2.8	101.8±11.6	26.6±3.9	5.8±0.4	396.2±39.2
Strain	(n=10)	29.8±2.3^f	179.0±11.7^{cf}	22.5±3.9^b	5.3±0.4^{bf}	354.0±29.7^{bf}
E_{low}	(n=10)	22.3±2.8^{cf}	221.9±19.0^{ce}	17.2±3.2^{ce}	4.3±0.3^{ce}	316.3±17.7^{ce}
E_{high}	(n=10)	61.9±12.4^{cf}	195.2±19.3^{cf}	20.9±2.9^{cf}	5.1±0.4^{cf}	331.3±23.4^{cf}
Oil	(n=10)	14.6±2.3^c	257.9±23.8^c	12.1±2.4^c	3.9±0.2^c	282.4±25.5^c

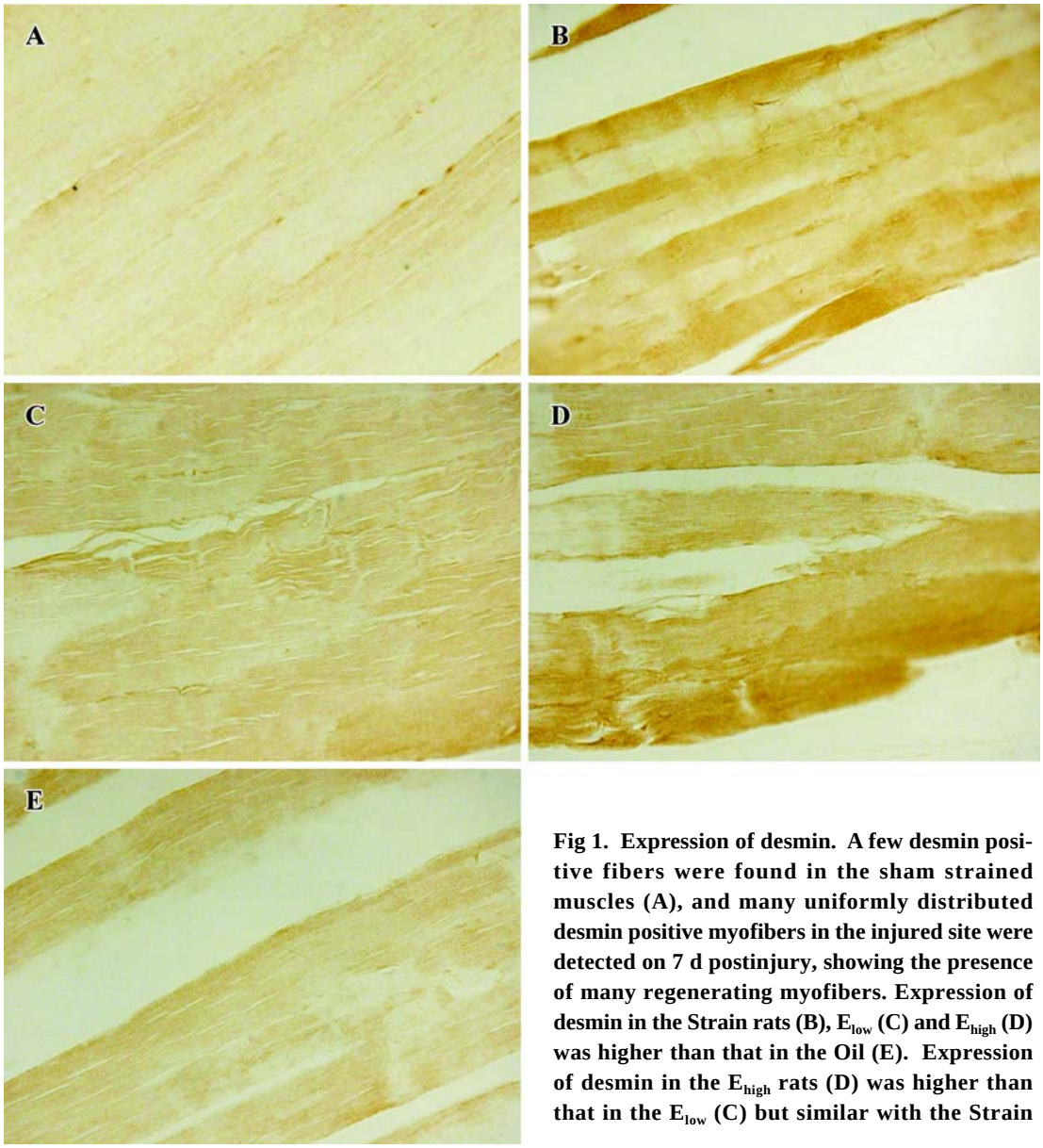

Fig 1. Expression of desmin. A few desmin positive fibers were found in the sham strained muscles (A), and many uniformly distributed desmin positive myofibers in the injured site were detected on 7 d postinjury, showing the presence of many regenerating myofibers. Expression of desmin in the Strain rats (B), E_{low} (C) and E_{high} (D) was higher than that in the Oil (E). Expression of desmin in the E_{high} rats (D) was higher than that in the E_{low} (C) but similar with the Strain

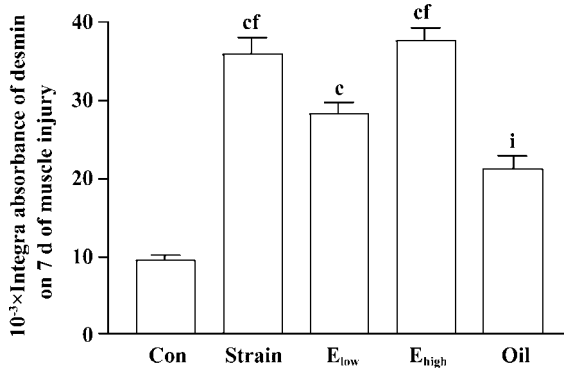

Fig 2. Integra absorbance of desmin-positive areas on 7 d of muscle strain injury in 5 groups. Mean±SD. $^{c}P<0.01$ *vs* Con. $^{f}P<0.01$ *vs* Oil. $^{i}P<0.01$ *vs* Strain.

tomized female rats.

We compared the differences of muscle damage among all the five groups. CK activity change has been used as indice of muscle damage in humans[33] and it was proposed that delayed rise in serum CK may be manifestation of secondary muscle damage[34]. The secondary muscle damage was mainly caused by oxygen-derived free radicals and lipid peroxidation followed by the ischemia and anoxia after the primary mechanical muscle injury[35,36]. In our experiments plasma CK activity increased after gastronemius strain injury, however CK activity in rats with intact ovaries or estrogen administration were apparently lower. These results indicated that estrogen could also act as membrane stabilizer to protect the skeletal muscle following acute strain

injury. GSH and Vit E are two important antioxidants and could greatly reduce the production of peroxide and lipid peroxidation so that stabilize the membrane and prevent the loss of enzymes from muscle fibers[37,38]. TAC was a global index of antioxidant protection and suitable not only as a protection against oxidation but also reflect consumption during acute oxidative processes[39]. In this study plasma Vit E, GSH, and TAC decreased after muscle strain injury due to their consumption following muscle injury, similar with the report of Bowles *et al*[19]. The Strain, E_{low}, or E_{high} group had higher Vit E, GSH and TAC contents compared with the Oil rats ($P<0.05$), which indicated that estrogen could decrease the consumption of Vit E and GSH, and maintain the TAC post muscle strain injury. From above, we think that estrogen could protect the muscle from primary mechanical stretch injury through membrane stabilization and from secondary muscle damage through anti-oxidation. The anti-oxidative effect of estrogen could be achieved partially by up-regulating the contents of GSH and Vit E to strengthen the total antioxidant capability *in vivo*.

Secondly, effects of estrogen on muscle regeneration were further observed. Muscle regeneration is the key process of muscle healing and here we chose desmin as the index of regeneration for its uniform expression in the regenerating myofibers[40,41]. As described by Stauber[42], we also observed low expression of desmin in the resting satellite cells of non-injured muscles and greatly increased expression post muscle injury, mainly locating in near area of Z disc during the muscle regenerating stage. It seems that rats with high plasma estrogen had high expression of the desmin and thus had accordingly high antioxidant ability and low CK activity. We supposed that estrogen could help facilitating muscle regeneration through its effects of protecting muscles from primary and secondary injury and maintaining the maximum muscle regeneration abilities. In addition, estrogen might activate skeletal muscle myoblast growth *in vitro*[43], so there may exist a possibility that estrogen could directly accelerate muscle regeneration, which needs further investigation.

Interestingly, we found that all the indexes were similar between the E_{high} and Strain group although plasma estradiol content of the E_{high} group was twofold greater than that of the Strain. Normal level of endogenous estrogen (physiological content) seemed to be enough to protect muscles from injury and improve regeneration. This may be ascribed to the limitation of amount of functional estrogen receptors *in vivo*.

In conclusion, both endogenous and exogenous estrogen could increase membrane stability, reduce the consumption of GSH and Vit E, and maintain total antioxidant capability *in vivo*, and consequently, decreased muscle injury and facilitated muscle regeneration post acute strain injury in the female rats.

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REFERENCES

1 Garrett WE. Muscle strain injuries: clinical and basic aspects. Med Sci Sports Exerc 1990; 22: 436-43.
2 Hughes C, Hasselman CT, Best TM, Martinez S, Garrett WE. Incomplete, intrasubstance strain injuries of the rectus femoris muscle. Am J Sports Med 1995; 23: 500-6.
3 McArdle A, Jackson MJ, editors. Intracellular mechanisms involved in skeletal muscle damage. New York: Oxford Medical Publications; 1997.
4 Nikolaou PK, MacDonald BL, Glisson RR, Seaber AV, Garrett WE. Biomechanical and histological evaluation of muscle after controlled strain injury. Am J Sports Med 1987; 15: 9-14.
5 Taylor DC, Dalton JD, Seaber AV, Garrett WE. Experimental muscle strain injury, early functional structural deficits and the increased risk for reinjury. Am J Sports Med 1993; 21: 190-4.
6 Pansarasa O, Castagna L, Colombi B, Vecchiet J, Felzani G, Marzatico F. Age and sex differences in human skeletal muscle: role of reactive oxygen species. Free Radic Res 2000; 33: 287-93.
7 Vignaud A, Noirez P, Besse S, Rieu M, Barritault D, Ferry A. Recovery of slow skeletal muscle after injury in the senescent rat. Exp Gerontol 2003; 38: 529-37.
8 Clarkson PM, Sayer SP. Practical and nutritional implications. In: Tarnopolsky MA, editor. Gender differences in exercise-induced muscle damage; v 7. Gender differences in metabolism. New York: CRC; 1999. p 283-300.
9 Komulainen J, Koskinen SO, Kalliokoski R, Takala TE, Vihko V. Gender differences in skeletal muscle fiber damage after eccentrically biased downhill running in rats. Acta Physiol Scand 1999; 165: 57-63.
10 Amelink GJ, Kamp HH, Bar PR. Creatine kinase isoenzyme profiles after exercise in the rat: sex-linked differences in leakage of CK-MM. Pflügers Arch 1988; 412: 417-21.
11 Amelink GJ, Bar PR. Exercise-induced muscle protein leakage in the rat. Effects of hormonal manipulation. J Neurol Sci 1986; 76: 61-68.
12 St Pierre Schneider B, Correia LA, Cannon JG. Sex differ-

ences in leukocyte invasion in injured murine skeletal muscle. Res Nurs Health 1999; 22: 243-50.
13 Stupka N, Tiidus PM. Effects of ovariectomy and estrogen on ischemia-reperfusion injury in hindlimbs of female rats. J Appl Physiol 2001; 91: 1828-35.
14 Tiidus PM, Bombardier E. Estrogen attenuates postexercise myeloperoxidase activity in skeletal muscle of malerats. Acta Physiol Scand 1999; 166: 85-90.
15 Tiidus PM, Holden D, Bombardier E, Zajchowski S, Enns D, Belcastro A. Estrogen effect on post-exercise skeletal muscle neutrophil infiltration and calpain activity. Can J Physiol Pharmacol 2001; 79: 400-6.
16 Carter A, Dobridge J, Hackney AC. Influence of estrogen on markers of muscle tissue damage following eccentric exercise. Fiziol Cheloveka 2001; 27: 133-7.
17 Delyani JA, Murohara T, Nossuli TO, Lefer AM. Protection from myocardial reperfusion injury by acute administration of 17beta-estradiol. J Mol Cell Cardiol 1996; 28: 1001-8.
18 Tiidus PM. Estrogen and gender effects on muscle damage, inflammation and oxidative stress. Can J Appl Physiol 2000; 25: 274-87.
19 Yagi K, Komura S. Inhibitory effect of female hormones on lipid peroxidation. Biochem Int 1986; 13: 1051-5.
20 Bowles DE, Torgan CE, Ebner S, Kehrer JP, Ivy JL, Starnes JW. Effects of acute, submaximal exercise on skeletal muscle vitamin E. Free Radic Res Commun 1991; 14: 138-43.
21 Sen CK, Atalay M, Agren J, Laaksonen DE, Roy S, Hanninen O. Fish oil and vitamin E supplementation in oxidative stress at rest and after physical exercise. J Appl Physiol 1997; l83: 189-95.
22 Kim YD, Farhat MY, Myers AK, Kouretas P, DeGroot KW, Pacquing A, *et al*. 17-Beta estradiol regulation of myocardial glutathione and its role in protection against myocardial stunning in dogs. J Cardiovasc Pharmacol 1998; 32: 457-65.
23 Tiidus PM. Oxidative damage, inflammation and muscle function. In: Tarnopolsky M, editor. Nutritional implications of gender differences in metabolism; v 27. Estrogen and oxygen radicals. Boca Raton: CRC; 1999. p 265-81.
24 Bornemann A, Schmalbruch H. Desmin and vimentin in regenerating muscles. Muscle Nerve 1992; 15: 14 -20.
25 Brocks L, Jap PH, Ramaekers FC, Stadhouders AM. Vimentin and desmin expression in degenerating and a regenerating dystrophic murine muscles. Virchows Arch B Cell Pathol Incl Mol Pathol 1991; 61: 89-96.
26 Singh M, Meyer EM, Millard WJ, Simpkins JW. Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats. Brain Res 1994; 644: 305-12.
27 Almeskinders LC, Gilbert JA. Healing of experimental muscle strains and the effects of nonsteroidal anti-inflammatory medication. Am J Sports Med 1986; 14: 303-8.
28 Obremsky WT, Seaber AV, Ribbeck BM, Garrett WE Jr. Biomechanical and histologic assessment of a controlled muscle strain injury treated with piroxicam. Am J Sports Med 1994; 22: 558-61.
29 Anderson ME. Enzymatic and chemical methods for the determination of glutathione. In: Dolphin D, editor. Glutathione. New York: Wiley; 1983. p 340-65.
30 Tietz F. Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Anal Biol Chem 1969; 27: 502.
31 Lee BL, Chua SC, Ong HY, Ong CN. High-performance liquid chromatographic method for routine determination of vitamin A and E and beta-carotene in plasma. J Chromatogr 1992; 581: 41-7.
32 Whitehead TP, Thorpe GH, Maxwell SR. An enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. Ann Clin Biochem 1997; 34: 412-21.
33 Jones A, Newham DJ, Round JM, Tolfree SE. Experimental human muscle damage: morphological changes in relation to other indices of damage. J Physiol 1986; 375: 435-48.
34 Faulkner JA, Brooks SV, Opiteck JA. Injury to skeletal muscle fibres during contractions: conditions of occurrence and prevention. Phys Ther 1993; 73: 911-21.
35 Duarte JA, Carvalho F, Bastos ML, Soares JM, Appell HJ. Do invading leucocytes contribute to the decrease in glutathione concentrations indicating oxidative stress in exercised muscle, or are they important for its recovery? Eur J Appl Physiol 1994; 68: 48-53.
36 Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement and significance. Am J Clin Nutr 1993; 57: 715-25.
37 Prem JT, Eppinger M, Lemmon G, Miller S, Nolan D, Peoples J. The role of glutamine in skeletal muscle ischemia/reperfusion injury in the rat hind limb model. Am J Surg 1999; 178: 147-50.
38 Meydani M, Evans WJ, Handelman G, Biddle L, Fielding RA, Meydani N, *et al*. Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. Am J Physiol 1993; 264: 992-8.
39 Lussignoli S, Fraccaroli M, Andrioli G, Brocco G, Bellavite P. A microplate-based colorimetric assay of the total peroxyl radical trapping capability of human plasma. Anal Biochem 1999; 269: 38-44.
40 Barash IA, Peters D, Friden J, Lutz GJ, Lieber RL. Desmin cytoskeletal modifications after a bout of eccentric exercise in the rat. Am J Physiol Regul Integr Comp Physiol 2002; 283: 958-63.
41 Vaittinen S, Lukka R, Sahlgren C, Hurme T, Rantanen J, Lendahl U, *et al*. The expression of intermediate filament protein nestin as related to vimentin and desmin in regenerating skeletal muscle. J Neuropathol Exp Neurol 2001; 60: 588-97.
42 Stauber WT, Smith CA. Cellular responses in exercise-induced skeletal muscle injury. Mol Cell Biochem 1998; 179: 189-96.
43 Kahlert S, Grohe C, Karas RH, Lobbert K, Neyses L, Vetter H. Effects of estrogen on skeletal myoblast growth. Biochem Biophys Res Commun 1997; 232: 373-78.