Impact of gingival inflammation on changes of a marker of muscle injury in young soccer players during training: A pilot study

Impacto da inflamação gengival sobre as alterações de marcador de lesão muscular em jovens jogadores de futebol durante treino: um estudo piloto

Bárbara Capitanio de Souza [a]
Marcelo Ekman Ribas [a]
Álvaro Reischak de Oliveira [b]
João Batista Burzlaff [c]
Alex Nogueira Haas [d]

[a] Faculty of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] School of Physical Education, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Dept. Orofacial and Maxillary Surgery, Faculty of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[d] Dept. Periodontology, Faculty of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

Abstract

Purpose: inflammatory markers involved in the etiopathogenesis of periodontal diseases are also found in muscle injury. The aim of the present study was to evaluate the impact of gingival inflammation on changes of serum levels of a marker of muscle injury (creatine kinase – CK) in young soccer players during regular training.

Methods: this observational longitudinal study included 15 male players (18±0.93 years of age). A calibrated examiner performed the periodontal examination in six sites per tooth in all fully erupted teeth, except third molars. Thereafter, blood samples for CK measurements (U/L) were taken at baseline, immediately and 20 hours after training.

Results: players presented poor oral hygiene (69.2% of sites with visible plaque). Bleeding on probing (BOP) scored 42.7% and probing depth (PD) averaged 2.3mm. CK levels changed significantly from 342.4 to 473.7 and 364.4 during the three time-points. A significant correlation was observed between PD and change in CK from the immediate measurement to 20h (r =-0.57). Significant correlations were observed between BOP and changes in CK from immediate measurement to 20h (r=-0.51) and from baseline to 20h (r=-0.52).

Conclusion: pocket depth and bleeding on probing were associated with changes in CK during training.

Key words: Creatine kinase; periodontal disease; muscle injury; athletes

Resumo

Objetivo: Marcadores inflamatórios envolvidos na etiopatogenia das doenças periodontais também são encontrados na lesão muscular. O objetivo do presente estudo foi avaliar o impacto da inflamação periodontal nas mudanças no nível sérico de um marcador de lesão muscular (creatina quinase – CQ) em jovens jogadores de futebol durante treinamento regular.

Metodologia: este estudo observacional longitudinal incluiu 15 jogadores do sexo masculino (18±0,93 anos de idade). Um examinador calibrado realizou exame periodontal de boca completa em seis sítios por dente em todos dentes permanente, exceto terceiros molares. Após, amostras sanguíneas para mensuração de CQ (U/L) foram obtidas no exame inicial, imediatamente e 20 horas apos treinamento.

Resultados: os jogadores apresentaram pobre higiene bucal (69,2% dos sítios com placa visível). Sangramento a sondagem (SS) foi registrado em 42,7% e profundidade de sondagem (PS) foi em média 2,3mm. Níveis de CQ mudaram significativamente de 342,4 para 473,7 e 364,4 durante os três tempos experimentais. Uma correlação significativa foi observada entre PS e mudança em CQ da mensuração imediata para 20 horas (r=-0,57). Correlações significativas foram observadas entre SS e mudanças em CQ da mensuração imediata para 20 horas (r=-0,51) e do exame inicial para 20 horas (r=-0,52).

Conclusão: profundidade de sondagem e sangramento a sondagem estiveram associados com mudanças em CQ durante treinamento.

Palavras chave: Creatina quinase; doença periodontal; lesão muscular; atletas

Correspondence:
Alex Nogueira Haas
Rua Ramiro Barcelos, 2492 – Periodontia
Bairro Bom Fim
Porto Alegre, RS – Brazil
90030-035
E-mail: alexnhaas@gmail.com

Received: August 6, 2012
Accepted: November 6, 2012

Conflict of Interests: The authors state that there are no financial and personal conflicts of interest that could have inappropriately influenced their work.

Introduction

Muscle injury is highly prevalent among athletes of various sports and has important negative impact on individual and collective performances (1). During the occurrence of a muscle lesion event, proteins are released to the blood as a result of the rupture of cell membrane (2), and measurements of serum enzymes have been used for screening of suspected muscle lesions. In this regard, creatine kinase (CK) is the most widely used screening protein because it has relative predominance in skeletal muscle, is not falsely elevated by hemolysis, and is readily released in cellular injury (3). Additionally, lactate (LAC) measurements have been used as metabolic parameter of effort intensity during training and competitions in various sport modalities (4).

Evidences have emerged that periodontal diseases may have an effect on systemic conditions, mainly cardiovascular diseases and adverse pregnancy outcomes (5). Moreover, it has been demonstrated that periodontal treatment can lead to changes in serum inflammatory biomarkers, such as C reactive protein and interleukins, involved in the occurrence of these conditions (6, 7). Some of these inflammatory markers are also involved in the biochemical cascade found during muscle injury (8). Moreover, there are personal reports of coaches from different sport modalities that athletes with poor oral health demonstrate lower physical performance (9). Consequently, on an inflamatory perspective, it is possible that periodontal inflamation may be associated with increased risk of muscle injury. Nevertheless, to the best of our knowledge, there are no studies published in the literature evaluating any association between periodontal diseases and muscle injuries.

The hypothesis of the present study is that athletes with higher levels of gingival inflammation will present higher elevation in serum levels of CK during training. Thus, the aim of the present pilot study was to evaluate the impact of gingival inflammation at baseline on changes of serum levels of CK in young soccer players during regular training.

Methodology

This study was an observational longitudinal study conducted in a local soccer team (Cruzeiro Sport Club) from Porto Alegre, Brazil. The study protocol *is in accordance with the Declaration of Helsinki*, and it has been reviewed and approved (protocol number 2008126) by the Research Ethics Committee of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil. All the subjects have read and signed a written informed consent. At the conclusion of the examination, the participants were provided with a written report detailing their oral status.

Male athletes, 14 years and older, who had at least 1 year of experience of training and at least 30 continuous days of regular soccer practice were included in this study. Additionally, to be included in the study, athletes should have permanent dentition with at least 20 teeth present. Smokers, individuals who used medications that can cause gingival hyperplasia or used systemic antibiotics in the last 3 months, who had muscle injuries under treatment or who have developed muscle lesions in the previous 60 days were excluded from the study. A total of 17 athletes were initially evaluated, whereas two were excluded from the study because they showed muscle lesions in less than 60 days. The demographic characteristics of the 15 individuals who participated in the study are shown in Table 1.

Interview and clinical examination

Participants answered a structured questionnaire regarding demographics, behavioral habits, overall health status and medication use. The periodontal condition was assessed by one trained and calibrated examiner. Visible plaque (VP), periodontal probing depth (PPD), gingival recession (GR) and bleeding on probing (BOP) were assessed in all participants in the Periodontal Clinic of the Faculty of Dentistry of the Federal University of Rio Grande do Sul. All permanent, fully erupted teeth, excluding third molars, were examined with a manual periodontal probe (Neumar, São Paulo, Brasil) color-coded at 1, 2, 3, 5, 7, 8, 9, 10 mm. Six sites per tooth were assessed at the mesiobuccal, midbuccal, distobuccal, distolingual, midlingual and mesiolingual sites.

First, VP was scored as present if a film of plaque was visible to the naked eye after drying the tooth with a blast of air. Consecutively, PPD and GR were assessed using a manual periodontal probe. Clinical attachment loss (CAL) was calculated as the sum of probing depth and gingival recession (or the difference between the two if the gingival margin was located coronal to the cementoenamel junction). BOP was recorded as present/absent after PPD measurements.

Creatine kinase and lactate

Blood samples were taken for measurements of creatine kinase and lactate. The serum level of CK was used as a marker of muscle injury, whereas the concentration of lactate was used as a marker of training intensity.

For the analysis of CK, 4mL of blood was collected from each participant from a vein in the antecubital region in three different times: before routine training (baseline), immediately after training (immediate) and 20 hours after training (20h). After sampling, blood was centrifuged in a refrigerated centrifuge (ALC PK 120 R, ALC International, Milan, Italy), at 5°C, with 3500rpm for 10 minutes. The serum was stored in microtubes, properly identified, which were preserved by cooling to -20°C until the time of analysis. Analysis of blood concentrations of CK was performed by dry chemistry and was expressed as U/L.

Measurements of lactate were made at two time-points: before training and during the final third of training. Blood samples for lactate analyses were obtained with a drop of venous blood from the tip of the index finger. Lactate concentrations were immediately assessed after sampling by a lactimeter (Accutrend® Roche, Basel, Switzerland) and expressed as mM.

Reproducibility

The examiner was trained and calibrated prior to the study. Intra-examiner clinical reproducibility was assessed after repeated periodontal examinations in 5 patients (744 sites) with clinical periodontal conditions similar to those from the present study. Reproducibility for PPD and CAL was evaluated by Kappa and intra-class correlation coefficients. Kappa values obtained for PPD and CAL were 0.72 and 0.57, respectively. Weighted kappa (±1mm) for PPD and CAL were 0.81 and 0.72, respectively. The intra-class correlation coefficients were 0.84 and 0.99, respectively.

Statistical analysis

Means of the periodontal parameters were calculated for each individual. Pearson correlation coefficients were estimated between inflammatory periodontal variables (PPD and BOP) and concentrations of CK in the different time-points of the study. Scatter plots with regression lines were used to illustrate the relationship between PPD, BOP and CK. R^2 values were also reported.

Individuals were categorized into high (≥47%) and low (<47%) BOP using the median of individual percentage of bleeding sites. This criterion was used arbitrarily, since there is no consensus in the literature regarding which is the best periodontal parameter or cut-off to define periodontal inflammation.

Clinical periodontal parameters were compared between high and low BOP groups using independent-samples t tests. Comparisons between the two BOP groups regarding the concentrations of CK over time were made using GLM (general linear model) repeated measures analysis of variance with the Bonferroni test for multiple comparisons.

Statistical analyses were performed using statistical software (SPSS 16 for Macintosh, SPSS Inc., Chicago Illinois). The individual was the unit of analysis and the significance level was set at 5%.

Results

Overall, study participants presented poor oral hygiene, as can be observed by the high percentage of sites with visible plaque (Table 1). No cases of periodontitis were diagnosed among the young soccer players in the present study. Periodontal destruction was very incipient (mean GR 0.16 mm), and overall PPD averaged 2.3 mm. On the contrary, more than 40% of the sites had bleeding on probing.

Periodontal parameters according to groups of higher and lower BOP are shown in Table 2. Mean BOP was 51.96% and 34.62% in groups with higher and lower BOP, respectively (P<0.001). The higher BOP group had significantly more gingival bleeding and pocket depth compared to the lower BOP group. No significant differences were observed between the two groups in regards to plaque and clinical attachment loss.

Table 1. Demographic and clinical characteristics of the sample at baseline.

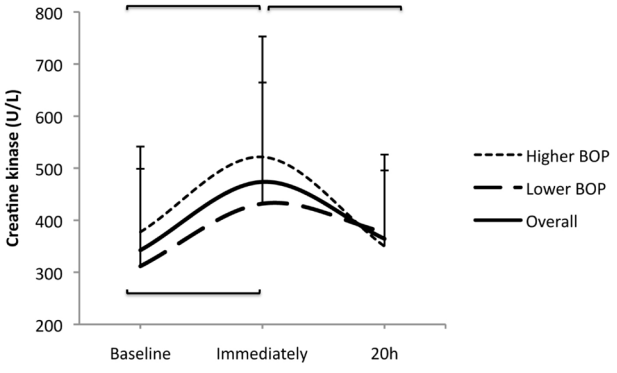
Variable	Estimate (N=15)
Age (average±SD)	18.0±0.93
Skin Color (N/%)	
White	8 (53.3)
Non-white	7 (46.7)
Education (N/%)	
High school incomplete	9 (60.0)
High school complete	6 (40.0)
Visible plaque (%, average±SD)	69.2±20.5
Probing pocket depth (mm, average±SD)	2.3±0.3
Gingival recession (mm, average±SD)	0.16±0.07
Bleeding on probing (%, average±SD)	42.7±11.2
Creatine kinase (U/L, average±SD)	342.4±173.8
Lactate (mM, average±SD)	1.8±0.6

Table 2. Periodontal parameters according to groups of high and low bleeding on probing.

	Higher BOP (≥47%) n=7	Lower BOP (<47%) n=8	*P**
Visible plaque (%)	78.74±7.63	60.77±24.92	0.09
PPD (mm)	51.96±11.72	32.29±15.99	0.02
CAL (mm)	2.47±0.26	2.16±0.22	0.03
Bleeding on probing (%)	0.14±0.06	0.18±0.08	0.26

* Independent samples t test.

Regarding muscle markers, the overall mean of lactate concentration before exercise was 1.8±0.6 mM, increasing to 4.2±2.6 mM at the final third of training. Overall baseline CK equaled 342.4 U/L for all soccer players (Figure 1, solid line). Immediately and 20 hours after training, overall CK levels changed to 473.7 U/L and 364.3 U/L, respectively. No significant difference in baseline CK levels was observed between groups of higher and lower BOP (377.4±164.2 U/L and 311.8±187.2 U/L, respectively; P=0.49).



Keys indicate significant difference over time within each group.
Absence of between-groups significant difference.

Figure 1. Mean (standard-deviation) concentration of creatine kinase (CK, in U/L) over time according to groups of bleeding on probing (BOP).

Table 3. Pearson correlation coefficients between inflammatory periodontal variables and CK at different time-points of physical exercise and between changes in CK (ΔCK).

	CK baseline		CK immediately after exercise		CK 20h		ΔCK baseline -immediately		ΔCK baseline -20h		ΔCK immediately -20h	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
PPD	0.42	0.12	**0.51**	**0.04**	0.24	0.39	0.5	0.06	-0.34	0.22	**-0.57**	**0.03**
BOP	0.09	0.74	0.13	0.66	-0.31	0.26	0.15	0.61	**-0.52**	**0.04**	**-0.51**	**0.04**

The repeated-measures general linear model did not showed between-groups difference (P=0.65), with a significant difference in CK over time (P<0.001), with no interaction between BOP and time (P=0.13). Overall CK changed significantly from baseline to the second examination (P<0.001) and from the second to the third examination (P=0.02). The same trend was observed in the two groups in CK changes after exercise, increasing immediately after training and decreasing after 20 hours. However, the reduction of CK immediately after exercise for 20 hours was statistically significant only among those with higher BOP.

Table 3 demonstrates Pearson correlation coefficients between PPD and CK levels and CK changes. There was a significant correlation between PPD and CK levels immediately after training (r=0.51). Regarding the correlation between PPD and changes in CK concentration, significant correlation was observed for changes from the immediate measurement to 20h (r =-0.57). In regards to bleeding on probing, significant correlations were observed only for changes in CK; for instance, from immediate measurement to 20h (r=- 0.51) and from baseline to 20h (r=-0.52).

Discussion

The present longitudinal study provided preliminary data to support an impact of the periodontal status on serum levels changes of a marker of muscle injury. The variation or the amount of change in CK levels during training was higher in young players with higher periodontal inflammation (higher BOP group). In addition, significant correlations were observed between PPD and BOP with changes in CK levels during training.

In general, soccer athletes participating in this study showed poor oral hygiene status, with high levels of plaque and gingival inflammation. These data are also reported in another article that rated football players in a similar age group (10). Contrarily, athletes presented low mean PPD and CAL and no cases of periodontitis were diagnosed. The increase in GI reflected gingival inflammation that may have been due to an increased bacterial accumulation and immune-system alterations affected by psychologic features, as also reported by other authors (11). These findings can be partly explained by the age of the participants. It is known that different age groups have different behaviors regarding oral health preventive practices. In addition, advanced periodontitis and/or CAL are rare in early age groups (12).

CK is an enzyme that catalyzes the transference of phosphate present in ATP (adenosine triphosphate) to creatine phosphate. It has high molecular weight and does not transpose the cell membrane under normal conditions, so it is considered sensitive for the diagnosis of muscular injury (13). Serum levels of CK may be used as markers of functional state of the muscular tissue, varying in specific physiological and pathological conditions. An increase of this enzyme may be an indicator of cellular necrosis and muscular tissue injury (14). For instance, CK levels in athletes are higher compared to non-athletes due to increased muscle mass and to intensive daily training. In non-athletes subjects, CK levels at rest reahes 150-160 U/L, (15) raising to 300-500 U/L after resistance exercises (16). CK values after exercise in trained athletes may reach peaks ranging from 975-1338 U/L (17). In this study, overall CK mean values were 342.4 U/L at rest (baseline), 473.7 U/L immediately and 364.3 U/L twenty hours after training. Baseline CK levels observed in this study are consistent with those reported in the literature for athletes and were higher than non-athletes subjects in general. Additionally, mean CK after training (473.7 U/L) was similar to that found by Lazarim et al. (17) in professional soccer players (493 U/L).

The blood LAC concentration has been frequently used as a metabolic parameter to quantify athletes' effort intensity during training. The average values after a strenuous physical activity such as a soccer game are 7.1 mM (18). Another study examined lactate levels in amateur athletes after a soccer game showing a range of 4 mM to 6 mM (19). Lactate levels found in elite soccer players range from 3 mM and 9 mM (20). In this study, it was observed an increase in lactate concentration form 1.8 mM to 4.2 mM, metabolically indicating that a moderate physical effort was performed.

Muscle injury resulting from physical exertion is common in sports that involve high-intensity physical activity, including soccer (1). An important aspect involving the relationship between inflammation and muscle injury is the role of inflammatory cells in the extent of tissue injury. It has been suggested that the inflammatory reaction can result in some local damage to healthy tissues (21). Inflammatory cytokines such as IL-6 and IL-8 are stimulated during the process of muscle damage and inflammation, having significant roles in stimulating neutrophil activity (22). Moreover, prolonged permanence of inflammatory cells and circulating pro-inflammatory mediators such as IL-1, IL-6, IL-8 and TNF-α may be responsible for collateral damage to healthy tissue as well. Changes in muscle cells

can significantly increase the expression of IL-1 and TNF-α, triggering an ongoing process of tissue damage. Animal models have demonstrated that when the neutrophil activity is controlled during chronic inflammation, tissue injury is attenuated by up to 40% (23). Furthermore, some studies suggest a dual role of interleukins (inflammatory and non-inflammatory) in the process of muscle damage and inflammation. However, a study using genetically engineered mice with over-expression of IL-6 found an association between increased levels of IL-6 and increased degradation of muscle proteins (24). These studies may indirectly indicate that the increase of inflammatory cytokines observed in the process of periodontal disease could influence the development and progress of muscle injuries.

The small number of participants in the present study may be considered one limitation. In this regard, the observed borderline p values may indicate lack of statistical power (higher observed power equaled 0.71). On the contrary, the strength of correlations (Table 3) indicates that an increased sample size would result in more evident associations. Another limitation of this study was the lack of standardization of physical effort by the athletes. However, lactate levels reached by the participating athletes indicate that there was a metabolic impact of the physical effort performed during training. Moreover, in these circumstances, it was possible to evaluate the impact of gingival inflammation in CK levels in regular and daily conditions of physical training. We could not find other studies that have evaluated the role of clinical periodontal inflammatory condition on biomarkers of muscle injury. As a consequence, no data is available for comparisons. Although this is a small study, this is the first time the relationship between gingival inflammation and increase of a well-established marker of muscle injury (CK) is assessed in a systematic manner. Moreover, the longitudinal design of our study allowed evaluating CK levels in different time-points and the impact of the periodontal condition on changes of this biomarker.

It can be concluded that PPD and BOP were associated with CK changes after exercise. Moreover, there seemed to be a greater impact of periodontal inflammation on changes of CK after exercise in individuals with higher BOP. These findings provide preliminary evidence on the association between oral and muscle health in young Brazilian soccer players, as well as raise the need for interventional studies to evaluate the impact of improving periodontal health on muscle injuries biomarkers. It is suggested that animal and intervention studies may be conducted to a better understanding of the possible influence of infectious and inflammatory periodontal status in the development and progress of muscle lesions.

Practioners of soccer and other sports should have special attention to oral care to maintain oral health and mainly to prevent and treat periodontal diseases since they may affect muscle metabolism.

References

1. Orchard J, Best TM. The management of muscle strain injuries: an early return versus the risk of recurrence. Clin J Sport Med 2002; 12: 3-5.
2. Sayers SP, Clarkson PM. Short-term immobilization after eccentric exercise. Part II: creatine kinase and myoglobin. Med Sci Sports Exerc 2003; 35: 762-768.
3. Brancaccio P, Limongelli FM, Maffulli N. Monitoring of serum enzymes in sport. Br J Sports Med 2006; 40: 96-97.
4. Silva PRS, Inarra LA, Vidal JRR, Oberg AARB, Fonseca Jr. A, Roxo CDalMN et al. Blood lactate levels in professional footballers checker after the first and second time in football matches. Acta Fisiátrica 2000; 7: 68-74.
5. Kinane D, Bouchard P. Periodontal diseases and health: Consensus Report of the Sixth European Workshop on Periodontology. J Clin Periodontol 2008; 35: 333-337.
6. Mustapha IZ, Debrey S, Oladubu M, Ugarte R. Markers of systemic bacterial exposure in periodontal disease and cardiovascular disease risk: a systematic review and meta-analysis. J Periodontol 2007; 78: 2289-2302.
7. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol 2008; 35: 277-290.
8. Peterson JM, Pizza FX. Cytokines derived from cultured skeletal muscle cells after mechanical strain promote neutrophil chemotaxis in vitro. J Appl Physiol 2009; 106: 130-137.
9. De Abreu DG. Mouth breathing and temporomandibular joint dysfunction (TMJ) orthodontic problems that can bring great damages to physical acting. Rev Bras Cienc Saúde 2009; 74-83.
10. Gay-Escoda C, Vieira-Duarte-Pereira DM, Ardèvol J, Pruna R, Fernandez J, Valmaseda-Castellón E. Study of the effect of oral health on physical condition of professional soccer players of the Football Club Barcelona. Med Oral Patol Oral Cir Bucal 2011; 16: 436-439.
11. Ulkar B, Elgun S, Ozmeric N, Özdemir B, Boynueğri D. Periodontal nitric oxide pathway alteration due to precompetition anxiety in handball players. J Periodontol 2012; 83: 204-210.
12. Susin C, Albandar JM. Aggressive periodontitis in an urban population in southern Brazil. J Periodontol 2005; 76: 468-475.

13. Susin C, Haas AN, Opermann RV, Albandar JM. Tooth loss in a young population from south Brazil. J Public Health Dent 2006; 66: 110-115.
14. Nosaka K, Newton M. Concentric or eccentric training effect on eccentric exercise-induced muscle damage. Med Sci Sports Exerc 2002; 34: 63-69.
15. Black HR, Quallich H, Gareleck CB. Racial differences in serum creatine kinase levels. Am J Med 1986; 81: 479-487.
16. Stromme JH, Rustad P, Steensland H, Theodorsen L, Urdal P. Reference intervals for eight enzymes in blood of adult females and males measured in accordance with the International Federation of Clinical Chemistry reference system at 37 degrees C: part of the Nordic Reference Interval Project. Scand J Clin Lab Invest 2004; 64: 371-384.
17. Lazarim FL, Antunes-Neto JM, da Silva FO, et al. The upper values of plasma creatine kinase of professional soccer players during the Brazilian National Championship. J Sci Med Sport 2009; 12: 85-90.
18. Silva PRS, Inarra LA, Vidal JRR, Oberg AARB, Fonseca Jr. A, Roxo CDalMN, Machado GS, Teixeira AAA. Blood lactate levels in professional footballers checker after the first and second time in football matches. Acta Fisiátrica 2000; 7: 68-74.
19. Gerisch G, Rutemoller E, Weber K. Sports Medical Measurements of Performance in Soccer. In: REILLY,T.; Lees A, Davies K, Murphy WJ. Science and Football 60-677, 1988.
20. Bangsbo J. The physiology of soccer-with special reference to intense intermittent exercise. Acta Physiol Scand 1994; Suppl 619: 1-155.
21. Tidball JG. Inflammatory cell response to acute muscle injury. Med Sci Sports Exerc 1995; 27: 1022-1032.
22. de Moura NR, Cury-Boaventura MF, Santos VC, Levada-Pires AC, Bortolon JR, Fiamoncini J et al. Inflammatory response and neutrophil functions in players after a futsal match. J Strength Cond Res (in press).
23. Jolly SR, Kane WJ, Hook BG, Abrams GD, Kunkel SL, Lucchesi BR. Reduction of myocardial infarct size by neutrophil depletion: effect of duration of occlusion. Am Heart J 1986; 112: 682-690.
24. Tsukinaka T, Ebisui C, Fujita J. Muscle undergoes atrophy in association with increase of lysosomal cathepsin activity in interleukin-6 transgenic mice. Biochem Biophys Res Commun 1995; 207: 168-174.