Reviews

Juntendo Medical Journal
2018. 64 (Suppl 1), 128–133

Evaluation of Chondroprotective Action of Glucosamine on Soccer and Rugby Players by Analyzing Type II Collagen Degradation and Synthesis Markers

Isao NAGAOKA*1), Akifumi TSURUTA*2), Masafumi YOSHIMURA*3)

*1) Department of Host Defense and Biochemical Research, Juntendo University Graduate School of Medicine, Tokyo, Japan, *2) Koyo Chemical Co. Ltd., Tokyo, Japan, *3) Juntendo University Graduate School of Health and Sports Science, Chiba, Japan

Cartilage and bone metabolism (type II collagen degradation) is increased by endurance exercise with intense joint loading. Interestingly, glucosamine-containing diet exhibits a chondroprotective action on osteoarthritis by inhibiting type II collagen degradation and improves the symptoms. Thus, in the present study, we evaluated the effect of glucosamine on cartilage metabolism in collegiate soccer players and professional rugby players with intense joint loading.

In soccer and rugby players, the urine level of type II collagen degradation maker CTX-II was significantly increased compared with non-athlete control, indicating that cartilage metabolism (type II collagen degradation) is increased in these athletes. In contrast, the urine level of type II collagen synthesis maker CPII was almost the same as in non-athletes. Based on these findings, the CTX-II/CPII ratios were higher in soccer and rugby players than non-athletes, suggesting that type II collagen degradation is relatively increased compared with type II collagen synthesis in these athletes. Importantly, the administration of glucosamine significantly decreased the CTX-II levels in soccer and rugby players; however, the CTX-II level returned to almost the pre-administration level after withdrawal of glucosamine administration. In contrast, the CPII level was not essentially changed during the test period. Based on these findings, the CTX-II/CPII ratios were reduced by glucosamine administration, and returned to the pre-administration level after withdrawal of glucosamine.

Together these observations suggest that glucosamine exhibits a chondroprotective action on endurance athletes, such as soccer and rugby players by preventing type II collagen degradation but maintaining type II collagen synthesis. However, the effect is transient and disappears after withdrawal of the administration.

Key words: glucosamine, cartilage metabolism, athletes, biomarker, type II collagen

Introduction

The frequency and severity of joint loading are critical factors for the development of joint destruction, characterized by the damage of articular cartilage. In fact, excessive loading on the joint with motion and exposure causes the damage of articular cartilage[1)-4)]. Thus, sports with repetitive impact and torsional loading on the joints increase the risk of articular cartilage degeneration, and results in the clinical symptoms of osteoarthritis[4)].

The disease process of osteoarthritis is related to the degradation and functional loss of articular cartilage. Importantly, the early changes in the metabolic and biochemical properties of cartilage matrix can be detected before the appearance of morphological changes of cartilage[2)]. Thus, various biomarkers have been developed as indicators of

Corresponding author: Isao Nagaoka
Department of Host Defense and Biochemical Research, Juntendo University Graduate School of Medicine
2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
TEL: +81-3-5802-1032 FAX: +81-3-3813-3157 E-mail: nagaokai@juntendo.ac.jp
〔**Received Oct. 23, 2017**〕〔Accepted Feb. 13, 2018〕

doi: 10.14789/jmj.2018.64.JMJ18-P52

cartilage and bone metabolism in subjects with joint and bone disorders[5]. In this context, it is interesting to note that sports-related mechanical loading on the joints affects the turnover rate of cartilage as well as bone in humans, and these changes can be detected by the assays with biomarkers[1)-4)].

Biomarkers for cartilage and bone metabolism

Type II collagen is one of the major components of cartilage[6], and the fragments of type II collagen are utilized as biomarkers for cartilage metabolism. A C-terminal telopeptide (CTX-II) is cleaved during degradation of type II collagen[7], whereas a neo-epitope (C2C) is cleaved at the C terminus of the 3/4 piece of degraded type II collagen[8]. Thus, both CTX-II and C2C are used as markers for type II collagen degradation. In contrast, a C-terminal type II procollagen peptide (CPII) is present in newly formed type II procollagen and cleaved during processing of synthesized type II procollagen; thus, CPII can be used as a marker for type II collagen synthesis[9]. In addition, deoxypyridinoline (Dpyr), a crosslink product of type I collagen and a cross-linked N-terminal telopeptide of type I collagen (NTx) are used as markers for type I collagen degradation in bone (bone resorption)[5].

Chondroprotective action of glucosamine on endurance athletes

Nutritional supplements, including glucosamine, chondroitin and collagen, are sometimes used for joint health to treat or prevent sports-related cartilage injuries (i.e., osteoarthritis) in athletes[10)-12)]. Among these, glucosamine, a naturally occurring amino monosaccharide, has been widely used to treat osteoarthritis in humans[13)-15)]. Thus, we looked at the chondroprotective action of glucosamine on athletes[16) 17)]. It has been already reported that sports and exercise affect cartilage and bone metabolism. O'Kane *et al.* compared the urine levels of type II collagen degradation marker CTX-II and type I collagen degradation marker NTx among non-athlete controls, cross-country runners, swimmers and crew members[3]. The results indicated that the levels of CTX-II and NTx are increased in the cross-country runners and crew members compared with non-athletes and swimmers, suggesting that cartilage and bone metabolism (type II and type I collagen degradation) is increased by endurance exercise with intense joint loading, such as cross county and boat racing.

So, we evaluated the effect of glucosamine on cartilage metabolism in collegiate soccer players with intense joint loading[16]. In this study, 10 non-athletes (23.5 ± 2.5 years old) and 21 soccer players (20.3 ± 0.9 years old) were recruited. Non-athletes experienced no hard exercise in the past year. In contrast, soccer players performed the training 5 days per week, and played the official match almost every weekend, during the test period.

Figures-1A and B show the urine levels of CTX-II and NTx in non-athlete controls and soccer players. In soccer players, the levels of CTX-II and NTx were significantly increased compared with non-athlete controls, indicating that cartilage and bone metabolism (type II and type I collagen degradation) is increased in soccer players, as reported in other endurance athletes[3]. Moreover, a type II collagen synthesis marker CPII was evaluated in soccer players. Interestingly, the urine level of CPII was slightly increased in soccer players compared with non-athlete controls (Figure-1C), suggesting that cartilage metabolism as evaluated by type II collagen synthesis may be increased in soccer players.

Further, we evaluated the type II collagen degradation and synthesis balance in the cartilage of soccer players by calculating CTX-II/CPII ratio. As shown in Figure-1D, the CTX-II/CPII ratio was significantly higher in soccer players than non-athlete controls, suggesting that type II collagen degradation is relatively increased compared with type II collagen synthesis in soccer players.

Next, we examined the effect of glucosamine administration on type II collagen degradation and synthesis markers. Importantly, the urine CTX-II level was significantly decreased after the glucosamine administration for 3 months at both 1.5 g and 3 g/day (Figure-2A). Interestingly, however, the CTX-II level returned to almost the pre-administration level after withdrawal of glucosamine administration in 1.5 g/day-group, although the CTX-II level was still reduced in 3 g/day-group. In contrast, the urine CPII level was not essentially

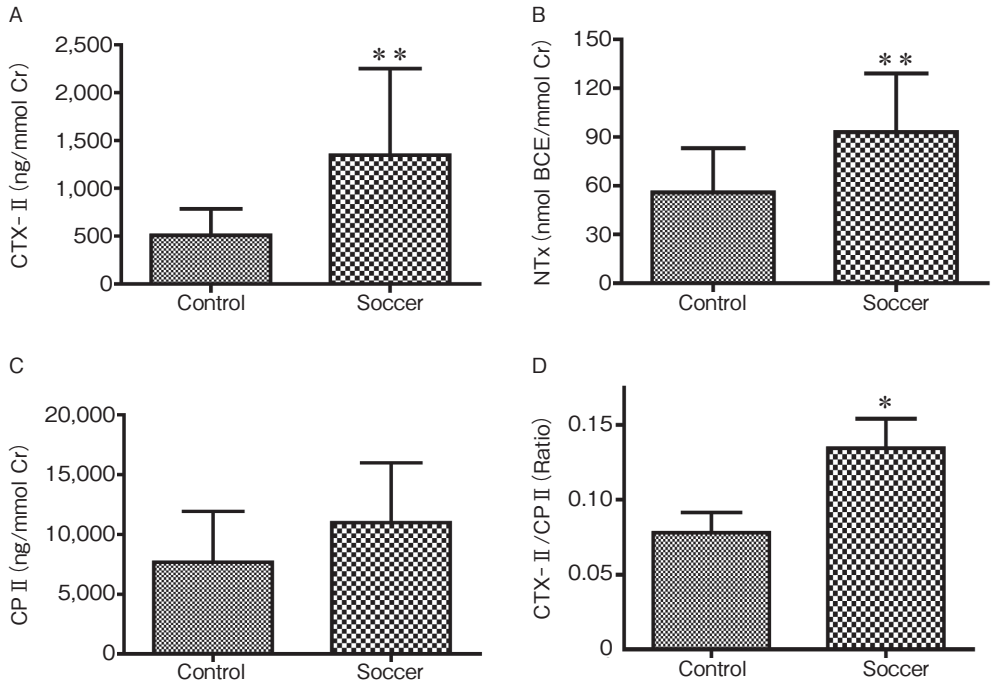

Figure-1 Comparison of the urine levels of CTX-II, NTx and CPII, and CTX-II/CPII ratio between non-athlete controls and soccer players

Urine levels of CTX-II (**A**), NTx (**B**) and CPII (**C**) in non-athlete controls and soccer players were measured by ELISA and corrected by urinary creatinine (Cr). Data represent the mean ± SD. Furthermore, the ratios of type II collagen degradation to synthesis (CTX-II/CPII) in non-athlete control and soccer players were calculated (**D**), using the levels of CTX-II and CPII shown in Figure 1A and C. Data represent the mean ± SEM. Values are compared between non-athlete controls (10 subjects) and soccer players (18 subjects). $^{*}p<0.05$, $^{**}p<0.01$

(Yoshimura M, *et al*: Int J Mol Med, 2009; 24: 487-494[16])

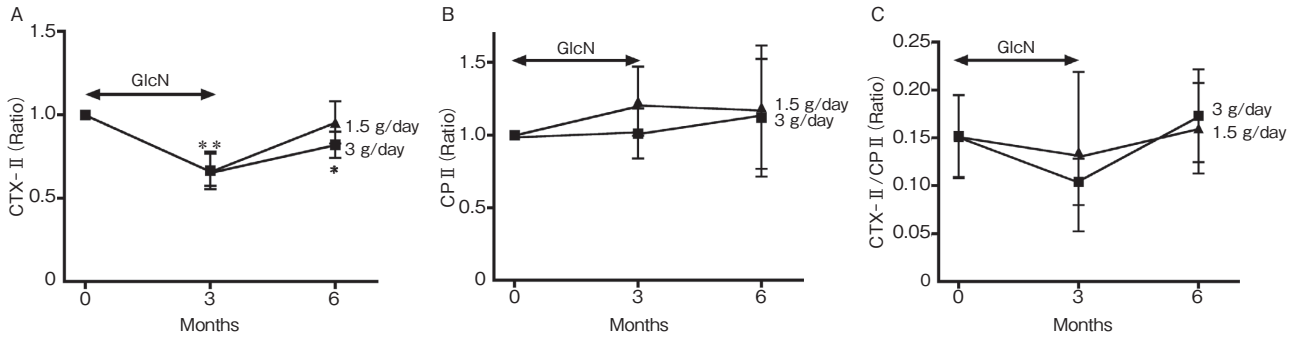

Figure-2 Effect of glucosamine administration on urine levels of CTX-II and CPII, and CTX-II/CPII ratio in soccer players

Soccer players were orally administered with glucosamine hydrochloride (GlcN; 1.5 or 3 g/day for 3 months, as indicated by an arrow). Urine levels of CTX-II (**A**) and CPII (**B**) were measured before (0 month), after the glucosamine administration (3 months) and after the withdrawal of glucosamine administration (6 months). These levels are expressed as a ratio relative to those before glucosamine administration (0 month). Furthermore, CTX-II/CPII ratios were calculated (**C**), using the levels of CTX-II and CPII before (0 month), after the glucosamine administration (3 months) and after the withdrawal of glucosamine administration (6 months). Data represent the mean ± SEM of 9 subjects (1.5 g GlcN/day) and 10 subjects (3 g GlcN/day). Values are compared between before (0 month) and after the glucosamine administration (3 months) or after the withdrawal of glucosamine administration (6 months). $^{*}p<0.05$, $^{**}p<0.01$

(Yoshimura M, *et al*: Int J Mol Med, 2009; 24: 487-494[16])

changed even after the glucosamine administration and withdrawal of glucosamine administration (Figure-2B), suggesting that the level of type II collagen synthesis in soccer players is maintained during the test period.

Furthermore, we evaluated the effect of glucosamine on type II collagen degradation and synthesis balance in soccer players by calculating CTX-II/CPII ratio. Importantly, the ratio was markedly reduced by glucosamine administration

especially at 3 g/day, and returned to the pre-administration level after withdrawal of glucosamine (Figure-2C).

These observations suggest that glucosamine exhibits a chondroprotective action in soccer players by preventing type II collagen degradation but maintaining type II collagen synthesis; however, its effect on type II collagen degradation is transient and disappears after withdrawal of administration.

Further, we evaluated the effect of glucosamine on cartilage metabolism in professional rugby players with intense joint loading[17]. In this study, 19 rugby players (29.4 ± 3.7 years old) and 19 non-athletes (29.4 ± 3.7 years old) were recruited[14]. Rugby players were administered everyday with a jelly-type diet containing 3 g glucosamine for 16 weeks.

Figures-3A and B show the urine levels of CTX-II and NTx in non-athletes and rugby players. In rugby players, the levels of CTX-II and NTx were significantly increased compared with non-athletes, indicating that cartilage and bone metabolism (type II and type I collagen degradation) is increased in rugby players, as reported in soccer players and other endurance athletes[3) 16)]. Next, we evaluated a type II collagen synthesis marker CPII in rugby players; however, the urine CPII level in rugby players was almost the same as in non-athletes (Figure-3C). Based on these findings, the CTX-II/CPII ratio was slightly higher in rugby players than non-athletes (Figure-3D), suggesting that type II collagen degradation is relatively increased compared with type II collagen synthesis in rugby players.

Next, we examined the effect of glucosamine administration on type II collagen degradation and synthesis markers. Importantly, the urine CTX-II level was significantly decreased after the glucosamine administration (Figure-4A). Interestingly, however, the CTX-II level returned to almost the pre-administration level after withdrawal of glucosamine administration. In contrast, the urine CPII level was not essentially changed even after the

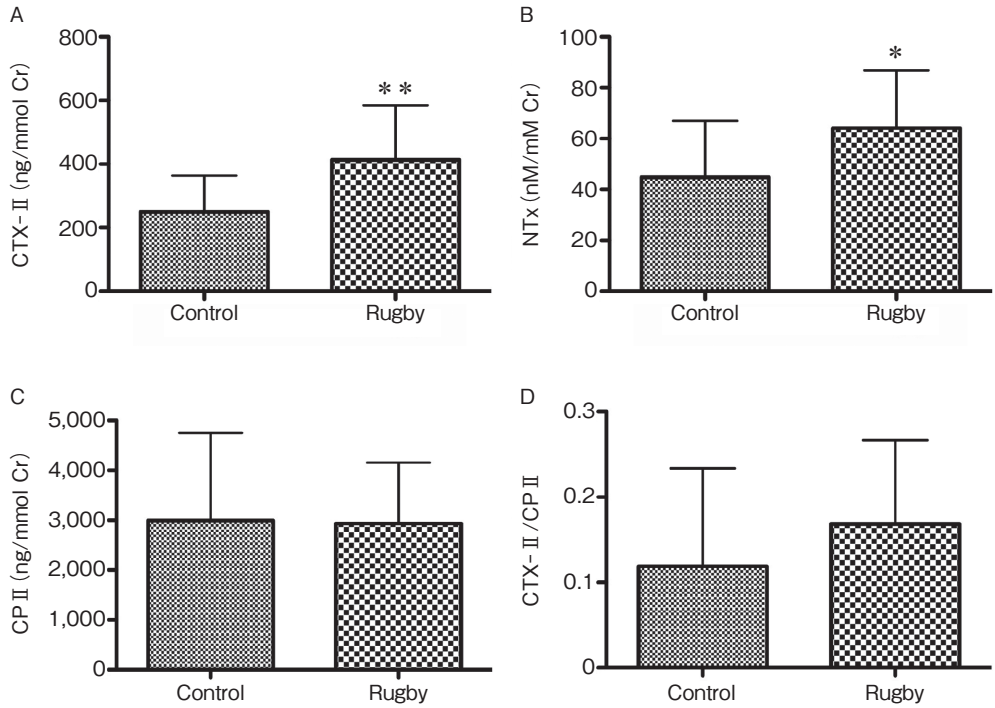

Figure-3 Comparison of the urine levels of CTX-II, NTx and CPII, and CTX-II/CPII ratio between non-athlete controls and rugby players

Urine levels of CTX-II (**A**), NTx (**B**) and CPII (**C**) in non-athlete controls and rugby players were measured by ELISA and corrected by urinary creatinine (Cr). Moreover, the ratios of type II collagen degradation to synthesis (CTX-II/CPII) in non-athlete controls and rugby players were calculated (**D**), using the levels of CTX-II and CPII shown in Figures 3A and C. Data represent the mean ± SD. Values are compared between non-athlete controls (19 subjects) and rugby players (19 subjects). $^{*}p<0.05$, $^{**}p<0.01$ (Tsuruta A, Nagaoka I: Functional Food Res, 2016; 12: 39-41[17])

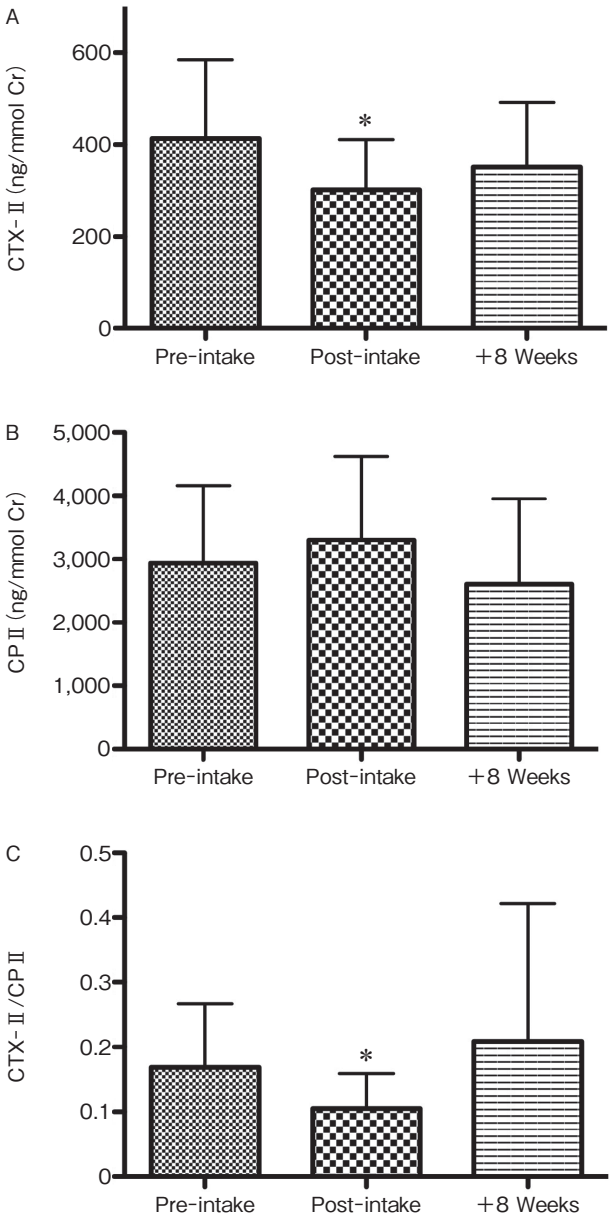

Figure-4 Effect of glucosamine administration on the urine levels of CTX-II and CPII, and CTX-II/CPII ratio in rugby players

Rugby players were administered everyday with a jelly-type diet containing 3 g glucosamine for 16 weeks. Urine levels of CTX-II (**A**) and CPII (**B**) were measured before (Pre-intake), after the glucosamine administration (Post-intake) and after the withdrawal of glucosamine administration (+8 weeks). Moreover, the ratios of type II collagen degradation to synthesis (CTX-II/CPII) were calculated using the levels of CTX-II and CPII before (Pre-intake), after the glucosamine administration (Post-intake) and after the withdrawal of glucosamine administration (+ 8 weeks) (**C**). Data represent the mean ± SD of 19 subjects. Values are compared between before (Pre-intake) and after the glucosamine administration (Post-intake). $^{*}p<0.05$.
(Tsuruta A, Nagaoka I: Functional Food Res, 2016; 12: 39-41 [17])

glucosamine administration and withdrawal of glucosamine administration (Figure-4B). Finally, we evaluated the effect of glucosamine on type II collagen degradation and synthesis balance in rugby players by calculating CTX-II/CPII ratio. Importantly, the ratio was significantly reduced by glucosamine administration, and returned to the pre-administration level after withdrawal of glucosamine (Figure-4C).

These observations suggest that glucosamine exhibits a chondroprotective action also in rugby players by preventing type II collagen degradation but maintaining type II collagen synthesis. However, the effect is transient and disappears after withdrawal of administration.

Conclusions

Type II collagen degradation is relatively increased compared with type II collagen synthesis in endurance athletes (soccer and rugby players). Glucosamine has been shown to inhibit the production of matrix metalloproteinase (MMP) -13 from chondrocytes and synoviocytes *in vitro* [18) 19)] and decrease the serum level of MMP-3 in sera of patients with rheumatoid arthritis [20)]. Based on these findings, it is interesting to speculate that glucosamine exhibits a chondroprotective action in endurance athletes (such as soccer and rugby players) by suppressing MMP production, thereby inhibiting type II collagen degradation (as evaluated by CTX-II) but maintaining type II collagen synthesis (as evaluated by CPII). However, the effect is transient and disappears after withdrawal of administration. Thus, glucosamine should be continuously administered for expecting joint health of endurance athletes.

References

1) Roos H, Dahlberg L, Hoerrner LA, *et al*: Markers of cartilage matrix metabolism in human joint fluid and serum: the effect of exercise. Osteoarthritis Cartilage, 1995; 3: 7-14.
2) Qi C, Changlin H: Effects of moving training on histology and biomarkers levels of articular cartilage. J Surg Res, 2006; 135: 352-363.
3) O'Kane JW, Hutchinson E, Atley LM, Eyre DR: Sport-related differences in biomarkers of bone resorption and cartilage degradation in endurance athletes. Osteoarthritis Cartilage, 2006; 14: 71-76.

4) Buckwalter JA, Lane NE: Athletics and osteoarthritis. Am J Sports Med, 1997; 25: 873–881.
5) Rousseau JC, Delmas PD: Biological markers in osteoarthritis. Nat Clin Pract Rheumatol, 2007; 3: 346–356.
6) Poole AR: Biochemical/immunochemical biomarkers of osteoarthritis: utility for prediction of incident or progressive osteoarthritis. Rheum Dis Clin North Am, 2003; 29: 803–818.
7) Christgau S, Garnero P, Fledelius C, *et al*: Collagen type II C–telopeptide fragments as an index of cartilage degradation. Bone, 2001; 29: 209–215.
8) Poole AR, Ionescu M, Fitzcharles MA, Billinghurst RC: The assessment of cartilage degradation in vivo: development of an immunoassay for the measurement in body fluids of type II collagen cleaved by collagenases. J Immunol Methods, 2004; 294: 145–153.
9) Shinmei M, Ito K, Matsuyama S, Yoshihara Y, Matsuzawa k: Joint fluid carboxy–terminal type II procollagen peptide as a marker of cartilage collagen biosynthesis. Osteoarthritis Cartilage, 1993; 1: 121–128.
10) Schwenk TL, Costley CD: When food becomes a drug: nonanabolic nutritional supplement use in athletes. Am J Sports Med, 2002; 30: 907–916.
11) Gorsline RT, Kaeding CC: The use of NSAIDs and nutritional supplements in athletes with osteoarthritis: prevalence, benefits, and consequences. Clin Sports Med, 2005; 24: 71–82.
12) Ostojic SM, Arsic M, Prodanovic S, Vukovic J, Zlatanovic M: Glucosamine administration in athletes: effects on recovery of acute knee injury. Res Sports Med, 2007; 15: 113–124.
13) McAlindon TE, Lavalley MP, Gulin JP, Felson DT: Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta–analysis. JAMA, 2000; 283: 1469–1475.
14) Reginster JY, Deroisy R, Rovati LC, *et al*: Long–term effects of glucosamine sulphate on osteoarthritis progression: a randomized, placebo–controlled clinical trial. Lancet, 2001; 357: 251–256.
15) Pavelká K, Gatterová J, Olejarová M, Machacek S, Giacovelli G, Rovati LC: Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3–year, randomized, placebo–controlled, double–blind study. Arch Intern Med, 2002; 162: 2113–2123.
16) Yoshimura M, Sakamoto K, Tsuruta A, *et al*: Evaluation of the effect of glucosamine administration on biomarkers for cartilage and bone metabolism in soccer players. Int J Mol Med, 2009; 24: 487–494.
17) Tsuruta A, Nagaoka I: Evaluation of a jelly–type functional food on the cartilage metabolism in male professional rugby players. Functional Food Res, 2016; 12: 39–41.
18) Nakamura H, Shibakawa A, Tanaka M, Kato T, Nishioka K: Effects of glucosamine hydrochloride on the production of prostaglandin E_2, nitric oxide and metalloproteases by chondrocytes and synoviocytes in osteoarthritis. Clin Exp Rheumatol, 2004; 22: 293–299.
19) Derfoul A, Miyoshi AD, Freeman DE, Tuan RS: Glucosamine promotes chondrogenic phenotype in both chondrocytes and mesenchymal stem cells and inhibits MMP–13 expression and matrix degradation. Osteoarthritis Cartilage, 2007; 15: 646–655.
20) Nakamura H, Masuko K, Yudoh K, Kato T, Kamada T, Kawahara T: Effects of glucosamine administration on patients with rheumatoid arthritis. Rheumatol Int, 2007; 27: 213–218.