Cartilage Metabolism in Endurance Athletes and Chondroprotective Action of Glucosamine

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In endurance athletes with intense joint loading, cartilage metabolism (degradation of type II collagen) is enhanced compared with non-athletes and non-endurance athletes. Recently, we have revealed that glucosamine, a functional food, exerts a protective action on cartilage metabolism in not only osteoarthritis patients but also endurance athletes (such as soccer players) by suppressing the degradation of type II collagen. In this review, to demonstrate these findings, the following topics will be presented: 1. Biomarkers for cartilage metabolism; 2. Evaluation of cartilage metabolism in endurance athletes by using biomarkers for cartilage metabolism; 3. Chondroprotective action of glucosamine on endurance athletes.

Key words: glucosamine, cartilage metabolism, biomarkers, joint health, endurance athlete

Introduction

The severity and frequency of joint loading are principal factors for the development of joint destruction, which is characterized by the articular cartilage damage. Actually, excessive motion and load on the joint cause the articular cartilage damage^{1)–4)}. Thus, sports with repetitive impact and torsional loading on the joints enhance the risk of articular cartilage degeneration, and result in the clinical symptoms of osteoarthritis⁴⁾. The pathological process of osteoarthritis leads to the degradation and functional loss of joint cartilage. Notably, the early changes of the cartilage metabolism can be detected before the appearance of morphological changes of cartilage²⁾. Thus, a number of biomarkers with reliability and sensitivity have been developed as indicators of cartilage metabolism in subjects with joint disorders⁵⁾.

Nutritional supplements, including glucosamine, chondroitin and collagen, are used for joint health to treat or prevent sports-related cartilage injuries

(i.e., osteoarthritis) in athletes^{6)–8)}. Among these, glucosamine, an amino monosaccharide, has been widely used to treat osteoarthritis in humans^{9)–12)}. Recently, we have revealed that glucosamine exhibits a protective action on cartilage metabolism in not only osteoarthritis patients but also endurance athletes by suppressing the degradation of type II collagen, as evidenced by the reduced level of type II collagen degradation markers^{13)–15)}. Thus, in this review, to demonstrate these findings, the following topics will be presented: 1. Biomarkers for cartilage metabolism; 2. Evaluation of cartilage metabolism in endurance athletes by using biomarkers for cartilage metabolism; 3. Chondroprotective action of glucosamine on endurance athletes.

Biomarkers for cartilage metabolism

Type II collagen is the major components of cartilage¹⁶⁾, and the fragments of type II collagen are utilized as biomarkers for cartilage metabolism (Figure-1). A C-terminal telopeptide (CTX-II) is

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Figure-1 Biomarkers for type II collagen degradation and synthesis

CTX-II and C2C are used as markers for type II collagen degradation. In contrast, CPII is used as a marker for type II collagen synthesis.

(Nagaoka I: Juntendo Medical Journal, 2017; 63: 104-114¹⁵⁾)

cleaved during degradation of type II collagen¹⁷⁾, whereas a neo-epitope (C2C) is cleaved at the C terminus of the 3/4 piece of degraded type II collagen¹⁸⁾. 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 synthesis¹⁹⁾. Moreover, type I collagen is the major components of bone; thus, deoxypyridinoline (Dpyr), a crosslink product of type I collagen and cross-linked N-terminal telopeptides of type I collagen (NTx) are used as markers for type I collagen degradation in bone

(bone resorption)⁵⁾.

Effect of endurance exercise on cartilage and bone metabolism

It has been 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 members³⁾. 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

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Table-1 Background of enrolled non-athletes and various sports athletes

		Ages (years)	Height (cm)	Weight (kg)	BMI (kg/m ²)
Non-athletes	n=10	21.1 ± 2.7	171.0 ± 6.0	62.9 ± 3.0	21.6 ± 1.3
Soccer	n=10	19.3 ± 0.7	176.2 ± 5.2	68.3 ± 5.8	22.0 ± 1.4
Tennis	n=9	19.8 ± 1.1	173.5 ± 5.2	67.9 ± 5.3	22.5 ± 1.4
Triathlon	n=10	20.3 ± 0.9	168.4 ± 2.5	62.0 ± 4.9	21.8 ± 1.3
Squash	n=11	20.8 ± 1.4	168.2 ± 5.8	63.2 ± 6.5	22.3 ± 1.9
Swimming	n=5	20.4 ± 0.9	179.8 ± 2.2	71.6 ± 4.2	22.1 ± 0.8
Volleyball	n=10	19.0 ± 0.7	183.4 ± 5.7	76.9 ± 6.5	22.8 ± 1.1
Kendo	n=9	20.4 ± 0.5	169.6 ± 2.3	66.0 ± 3.6	22.9 ± 1.2
Handball	n=10	18.8 ± 0.6	172.7 ± 5.5	66.5 ± 6.0	22.3 ± 1.9
Baseball	n=10	19.5 ± 1.1	172.6 ± 6.1	69.8 ± 4.8	23.4 ± 1.0
Gymnastics	n=10	20.5 ± 1.4	166.2 ± 4.7	60.7 ± 5.0	22.0 ± 1.1
Judo	n=10	18.7 ± 0.7	171.4 ± 5.6	79.7 ± 13.8	27.0 ± 3.5
Basketball	n=10	20.3 ± 1.3	181.9 ± 8.0	76.1 ± 7.9	23.0 ± 1.3
Long-distance	n=10	20.5 ± 1.2	167.3 ± 6.3	53.1 ± 4.3	19.0 ± 0.6
Sprint	n=7	18.6 ± 0.5	173.3 ± 4.9	64.6 ± 5.0	21.5 ± 1.0
Jumping	n=10	19.2 ± 0.8	175.6 ± 4.3	65.1 ± 6.0	21.1 ± 1.4
Throwing-event	n=9	18.8 ± 0.8	178.0 ± 4.6	94.7 ± 10.4	29.9 ± 3.0
Futsal	n=10	19.0 ± 0.9	171.1 ± 4.5	64.8 ± 4.5	22.2 ± 1.5

Data represent the mean ± SD.

(Nagaoka I, Tsuruta A, Yoshimura M: Juntendo Medical Journal, 2018; 64 (Suppl 1): 122-127²⁰⁾)

and bone metabolism (type II and type I collagen degradation) is increased by endurance exercise with intense joint loading, such as cross country and boat racing.

Thus, to test this hypothesis, we evaluated the cartilage and bone metabolism in collegiate athletes belonging to various sports clubs (soccer, tennis, triathlon, squash, swimming, volleyball, kendo, judo, gymnastics, basketball, handball, baseball, long-distance, throwing-event, jumping, sprint and futsal) by analyzing the urine levels of type II collagen degradation marker CTX-II and synthesis marker CPII, and type I collagen degradation marker NTx, and compared with those of non-athlete controls²⁰⁾.

Table-1 shows the background of enrolled non-athlete controls and athletes (all males). First, NTx levels were compared among athletes of various sporting events. The results indicated that NTx levels were significantly higher in soccer, volleyball, basketball and handball players than non-athlete controls (Figure-2).

Furthermore, the levels of CTX-II and CPII were compared between non-athlete controls and athletes of various sporting events. Similar to the levels of NTx, CTX-II levels were significantly higher in soccer, volleyball and handball players than non-

athlete controls (Figure-3A). In contrast, CPII levels were significantly higher in squash players and long-distance runners than non-athlete controls (Figure-3B). Based on the levels of CTX-II and CPII, the CTX-II/CPII ratios were calculated and compared between non-athlete controls and athletes of various sporting events. The results indicated that the CTX-II/CPII ratios were higher in soccer, volleyball, basketball and handball players than non-athlete controls (Figure-3C), suggesting that type II collagen degradation is relatively enhanced compared with type II collagen synthesis in these athletes.

The similar changes of NTx (Figure-2) and CTX-II (Figure-3A) among various sports athletes indicate that NTx and CTX-II are likely to be changed in parallel within the body, although the two markers represent different bone and cartilage metabolism (type I collagen degradation and type II collagen degradation, respectively)⁵⁾. To confirm this, we analyzed the correlation between the levels of NTx and CTX-II. As expected, the levels of NTx and CTX-II were significantly correlated (Figure-4A). In contrast, there was no significant correlation between NTx and CPII (Figure-4B), and CTX-II and CPII (Figure-4C). These observations

Figure-2 Comparison of the urine levels of NTx between non-athlete controls and various sports athletes. Urine levels of NTx in non-athlete controls and various sports athletes (shown in Table-1) were measured by ELISA and corrected by urinary creatinine (Cr). Data represent the mean $\pm$ SD. Values are compared between non-athletes (10 subjects) and various sports athletes (5~11 subjects) by one-way ANOVA with Bonferroni post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(Nagaoka I, Tsuruta A, Yoshimura M: Juntendo Medical Journal, 2018; 64 (Suppl 1): 122-127²⁰⁾)

indicate that type II collagen degradation (as assessed by CTX-II) is correlated with type I collagen degradation (as assessed by NTx) in sports athletes, and support our hypothesis that although both NTx and CTX-II are regarded as different bone and cartilage markers⁵⁾, their levels are changed in parallel within the body.

Chondroprotective action of glucosamine on endurance athletes

Next, we evaluated the chondroprotective action of glucosamine on endurance exercise. Glucosamine is an amino monosaccharide with an amino group, and functions as a component of glycosaminoglycans (such as hyaluronic acid and chondroitin sulfate)²¹⁾ (Figure-5). Figure-6 shows the synthetic pathway of glycosaminoglycans in our body²²⁾; glucose is converted into glucosamine with the addition of amino group from glutamine, and further converted into N-acetyl-glucosamine and N-acetyl-galactosamine. Then, these glucosamine-derivatives are coupled with uronic acid to form glycosaminoglycans (such as hyaluronic acid,

keratin sulfate and chondroitin sulfate) present in the articular cartilage, skin and other tissues. Importantly, glucosamine supplement is efficiently absorbed from the intestine, distributed to various tissues and used as a constituent of glycosaminoglycans. Thus, glucosamine is widely utilized as a functional food with a chondroprotective action to treat human diseases such as osteoarthritis as a precursor of glycosaminoglycans⁹⁾⁻¹²⁾.

Furthermore, we have demonstrated that glucosamine suppresses the activation of neutrophils²³⁾, synovial cells²⁴⁾ and intestinal epithelial cells²⁵⁾, endothelial cells²⁶⁾, and inhibits adjuvant arthritis²⁷⁾, colitis²⁸⁾ and atherosclerosis²⁹⁾ in animal models. Thus, glucosamine expectantly exhibits anti-inflammatory actions.

So, we evaluated the effect of glucosamine on cartilage metabolism using collegiate soccer players with intense joint loading³⁰⁾. In this study, 41 soccer players (mean age, 20.2 ± 1.1 years; 20 subjects in the placebo group, 21 subjects in the glucosamine group) were recruited. Subjects were randomly assigned to receive a 2,000 mg glucosamine-containing supplement (glucosamine group) or a

Figure-3 Comparison of the urine levels of CTX-II and CPII, and CTX-II/CPII ratio between non-athlete controls and various sports athletes

Urine levels of CTX-II (A) and CPII (B) in non-athlete controls and various sports athletes (shown in Table-1) 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 various sports athletes were calculated (C), using the levels of CTX-II and CPII shown in Figures 3A and B. Data represent the mean $\pm$ SD. Values are compared between non-athletes (10 subjects) and various sports athletes (5–11 subjects) by one-way ANOVA with Bonferroni post hoc test. * $p < 0.05$, ** $p < 0.01$.

(Nagaoka I, Tsuruta A, Yoshimura M: Juntendo Medical Journal, 2018; 64 (Suppl 1): 122–127²⁰⁾)

Figure-4 Correlation analysis of bone and cartilage metabolism markers

Correlations between CTX-II and NTx (A), CPII and NTx (B), and CPII and CTX-II (C) were analyzed among enrolled subjects ($n=170$, non-athletes controls and various sports athletes, shown in Table-1).

(Nagaoka I, Tsuruta A, Yoshimura M: Juntendo Medical Journal, 2018; 64 (Suppl 1): 122-127²⁰⁾)

placebo containing only a vehicle (placebo group). All subjects were instructed to take the test supplement or placebo once a day within 30 min after exercise for 16 weeks.

Table-2 presents the baseline characteristics of these subjects, including age, physiological characteristics (body height, body weight and body mass index) and levels of biomarkers for type I collagen

metabolism (NTx) and type II collagen metabolism (CTX-II, C2C and CPII). There were no significant differences in these parameters between the placebo and glucosamine groups at the baseline.

The effect of test supplement on cartilage metabolism was evaluated using these subjects at weeks 0 and 16 during the intervention. Notably, urine CTX-II level significantly decreased in the glucosamine group but not in the placebo group after the intervention for 16 weeks ($p<0.05$); moreover, CTX-II levels were significantly decreased in the glucosamine group compared with that in the placebo group after the intervention for 16 weeks ($p<0.05$) (Figure-7A). Similarly, serum C2C level significantly decreased in the glucosamine group but not in the placebo group after the intervention for 16 weeks ($p<0.05$), although C2C levels were not significantly different between the placebo and glucosamine groups after the intervention for 16 weeks (Figure-7B). In contrast, urine CPII levels were not significantly different between weeks 0 and 16 during the intervention in the placebo or glucosamine group, and between the placebo and glucosamine groups after the intervention for 16 weeks (Figure-7C). Similarly, urine NTx levels were not significantly different between weeks 0 and 16 during the intervention in the placebo or glucosamine group, and between the placebo and glucosamine groups after the intervention for 16 weeks (Figure-7D).

Discussion and Conclusion

Cartilage biomarkers can be used to evaluate the pathophysiological conditions of joint disorders⁵⁾. The biomarkers are basically derived from the constituents of cartilage, such as aggrecan, chondroitin sulfate and collagens^{5) 16)}. Among these constituents, type II collagen is a major constituent of articular cartilage, and the catabolism and anabolism of articular type II collagen are involved in the pathological conditions of joint disorders; thus, the components of type II collagen are recognized as the most important biomarkers for cartilage metabolism and joint disorders (such as osteoarthritis)³¹⁾. In this review, I presented the data on the cartilage and bone metabolism in college athletes belonging to various sports clubs, which were analyzed based on the levels of type II

Figure-5 Structures of glucosamine and glycosaminoglycans

Show are the basic structures of glucosamine and glycosaminoglycans such as hyaluronic acid and chondroitin sulfate, which are composed of uronic acid (such as glucuronic acid) and glucosamine-derived monosachharides (such as N-acetyl-glucosamine and N-acetyl-galactosamine). Glucosamine is converted from glucose by the addition of amino group from glutamine. (Nagaoka I: Juntendo Medical Journal, 2014; 60: 580-587¹²⁾)

Figure-6 Synthesis of glycosaminoglycans from glucosamine-derivatives and uronic acid (Nagaoka I: Juntendo Medical Journal, 2014; 60: 580-587¹²⁾)

Table-2 Baseline characteristics of the subjects in the placebo and glucosamine groups

	Placebo group (n=20)	Glucosamine group (n=21)	p-value
Ages (years)	20.2 ± 1.2	20.2 ± 1.0	0.45
Height (cm)	173.0 ± 5.5	175.1 ± 5.1	0.70
Weight (kg)	67.1 ± 6.0	69.0 ± 5.3	0.54
BMI (kg/cm ²)	22.4 ± 1.1	22.5 ± 1.2	0.85
NTx (nmol BCE/mmol Cr)	101.9 ± 25.0	77.9 ± 26.9	0.76
CTX-II (ng/mmol Cr)	1,510.1 ± 1,038.0	1,250.7 ± 674.1	0.06
CPII (ng/mmol Cr)	3,356.6 ± 1,892.6	2,826.8 ± 2,038.8	0.75
C2C (ng/ml)	22.3 ± 7.2	20.7 ± 9.7	0.20

Values are expressed as the mean ± SD. Baseline characteristics of subjects were analyzed by Student's t-test between the placebo and glucosamine groups.

BMI, body mass index; NTx, type I collagen degradation marker; CTX-II and C2C, type II collagen degradation markers; CPII, type II collagen synthesis marker.

Figure-7 Effect of glucosamine administration on the biomarkers for type II collagen degradation, type II collagen synthesis and type I collagen degradation in soccer players

Soccer players (shown in Table-2) were orally administered with a placebo or glucosamine-containing supplement (2,000 mg/day for 16 weeks), and urine and serum samples were collected at weeks 0 and 16 during the glucosamine administration. Urine CTX-II (A), serum C2C (B), urine CPII (C) and urine NTx (D) were measured by ELISA, and the levels of urine markers were corrected by urinary creatinine (Cr). Data are the mean ± SD of 20 subjects in the placebo group and 21 subjects in the glucosamine group. Values were compared between weeks 0 and 16 in the placebo or glucosamine groups, and among multiple groups (such as the placebo and glucosamine groups at weeks 0 and 16 during the intervention) by two-way ANOVA with Tukey post hoc test. *p < 0.05, **p < 0.01. (Tsuruta A, Horiike T, Yoshimura M, Nagaoka I: Mol Med Reports, 2018; 18: 3941-3948³⁰⁾)

collagen degradation marker CTX-II and synthesis marker CPII, and type I collagen degradation marker NTx²⁰⁾. The results indicated that cartilage metabolism (type II collagen degradation) as well as bone metabolism (type I collagen degradation) is enhanced in endurance exercise associated with jumping action, such as soccer, volleyball, basketball and handball.

Further, I presented the data on the chondroprotective action of glucosamine on endurance athletes, especially soccer players with intense joint loading³⁰⁾. The results revealed that urine CTX-II level significantly decreased in the glucosamine group but not in the placebo group after the intervention for 16 weeks ($p < 0.05$) (Figure-7A). Similarly, serum C2C level significantly decreased in the glucosamine group but not in the placebo group after the intervention for 16 weeks ($p < 0.05$) (Figure-7B). In contrast, the levels of urine CPII as well as urine NTx were not significantly changed even after the intervention in both the placebo and glucosamine group (Figure-7C and D). Thus, glucosamine administration (2,000 mg/day for 16 weeks) significantly reduces the levels of CTX-II and C2C but not CPII and NTx, confirming that glucosamine exerts a chondroprotective action in endurance athletes (soccer players) by suppressing type II collagen degradation (as assessed by CTX-II and C2C, type II collagen degradation markers).

It has been reported that glucosamine suppresses the production of matrix metalloproteinase (MMP)-13, a major type II collagen-degrading enzyme, from chondrocytes and synoviocytes *in vitro*^{32) 33)} and reduces the serum level of MMP-3 in sera of patients with rheumatoid arthritis³⁴⁾. Based on these findings, it is interesting to speculate that glucosamine inhibits MMP production, thereby suppressing type II collagen degradation (as evidenced by the reduction of CTX-II and C2C levels) *in vivo*. In contrast, glucosamine administration did not essentially affect the levels of CPII as well as NTx, suggesting no effect of glucosamine administration on the levels of type II collagen synthesis (CPII) and type I collagen degradation (NTx) in soccer players. Importantly, glucosamine has been reported to enhance the expression of type II collagen in chondrocytes *in vitro*³³⁾; however, the increase of type II collagen synthesis (as

evaluated by CPII) could not be detected in soccer players (Figure-7C). This is probably due to the fact that type II collagen synthesis (as evaluated by CPII) was slightly increased in soccer players compared with non-athlete controls (Figure-3B)^{14) 20)}, although the increase is not significant; thus, the CPII level cannot be further enhanced by glucosamine administration.

In summary, type II collagen degradation is relatively increased compared with type II collagen synthesis in endurance athletes. Glucosamine exhibits a chondroprotective action in endurance athletes (such as soccer players) by preventing type II collagen degradation but maintaining type II collagen synthesis.

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