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BONE METABOLISM MARKERS IN SPORTSWOMEN WITH MENSTRUAL CYCLE DYSFUNCTIONS

MARKERI KOŠTANOG METABOLIZMA KOD SPORTISTKINJA SA POREMEĆAJIMA MENSTRUALNOG CIKLUSA

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Summary: It is a well known fact that sportswomen with irregular menstrual cycle are exposed to the risk of diminished bone mineral density, and consequentially osteoporosis may appear. Monitoring of the levels of biochemical markers of bone metabolism enables understanding of the dynamic changes during the bone remodeling process. The objectives of the conducted research were to determine the prevalence of menstrual dysfunctions in a sportswomen sample and a control group, and also to determine the levels of bone metabolism markers in groups of women with menstrual dysfunctions. The women (n=117) were separated into two groups, the experimental group (S) (n=84) comprised of three subgroups of sportswomen (34 women who play ball game sports, 27 athletes and 23 sport dancers) and the control group (C) (n=34). To establish the menstrual profile and dysfunction of the menstrual cycle, we used a very detailed questionnaire. The level of mid-fragment osteocalcin (N-MID osteocalcin) as a marker of bone formation was determined, as well as β-CrossLaps (β-CTx–bone resorption marker) via the electroluminescent immunochemistry method on an Elecsys 1010 automated machine. Primary amenorrhea was found in 7 (8.33%) and oligomenorrhea in 11 (13.09%) sportswomen, which was statistically a much higher incidence ($p<0.05$) than in the control group (0/34). Values of bone metabolism markers showed a statistically significant difference in the level of the bone resorption marker β-CrossLaps between the groups of amenorrheic and oligomenorrheic sportswomen in comparison to the eumenorrheic women, both sportswomen and

Kratak sadržaj: Dobro je poznata činjenica da su sportistkinje sa neredovnim menstrualnim ciklusom izložene riziku od smanjenja koštane mineralne gustine i posledično osteoporozi. Praćenje nivoa biohemijskih markera koštanog metabolizma omogućava razumevanje dinamičkih promena tokom procesa remodeliranja kosti. Ciljevi sprovedenog istraživanja bili su: utvrditi prevalence menstrualnih poremećaja na uzorku sportistkinja i kontrolne grupe, kao i odrediti nivoe markera koštanog metabolizma u grupama ispitanica sa menstrualnim disfunkcijama. Ispitanice (n=117) bile su podeljene u dve grupe, eksperimentalnu (S) (n=84) podeljenu u tri podgrupe (34 sportistkinje igara sa loptom, 27 atletičarki i 23 takmičarke u sportskom plesu) i kontrolnu grupu (C) (n=34). Za određivanje menstrualnog profila i poremećaja menstrualnog ciklusa korišćen je upitnik. Određen je nivo srednjeg fragmenta osteokalcina (N-MID osteokalcin) kao markera formiranja kosti i β-CrossLaps (β-CTx – marker resorpcije kosti) elektroluminiscentnom imunohemijskom metodom na automatskom aparatu Elecsys 1010. Primarna amenoreja nađena je kod 7 (8,33%) a oligomenoreja kod 11 (13,09%) sportistkinja, što je statistički značajno viša incidenca u odnosu na kontrolnu grupu. Vrednosti markera koštanog metabolizma su pokazale statistički značajnu razliku u nivou markera resorpcije kosti, β-CrossLaps, između grupa amenoreičnih i oligomenoreičnih sportistkinja u odnosu na eumenoreične ispitanice, kako sportistkinje tako i kontrolnu grupu. Ubrzanu resorpciju je pratilo i ubrzano formiranje kosti (povećane

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List of abbreviations: BMD – bone mineral density; OC (N-MID osteocalcin) – serum osteocalcin; β-CTx (β-CrossLaps) – serum C terminal cross-linked telopeptide of type I collagen; ECLIA – electroluminescent immunochemistry method; ACSM – American College of Sports Medicine; HPA – Functional hypothalamic amenorrhea.

those in the control group. Accelerated resorption was accompanied with accelerated bone formation. Menstrual dysfunctions were statistically more present in the sportswomen group than in the control group and were accompanied with accelerated bone metabolism from the point of view of the increase of bone metabolism markers level.

Keywords: bone remodeling markers, β-CTx, functional hypothalamic amenorrhea, osteocalcin, premature osteoporosis

vrednosti osteokalcina). Menstrualne disfunkcije statistički su značajno više prisutne u grupi sportistkinja u odnosu na kontrolnu grupu i, gledajući nivoe markera metabolizma kosti, bile su praćene ubrzanim metabolizmom kosti.

Ključne reči: β-CTx, hipotalamička funkcionalna amenoreja, markeri metabolizma kosti, osteokalcin, prevremena osteoporoza

Introduction

Bone is a metabolically active tissue, and bone remodeling consisting of bone formation and resorption is a process that goes on throughout life. The balance between these two processes enables the maintaining of the overall bone mass and the morphological structure of the bone (1). At least 60–70% of the maximum of bone mass is generated during puberty and adolescence through the so-called »window of opportunity«, and up to 90% by the end of the second life decade (2, 3). Different forms of physical activity positively affect the reaching of the peak of bone mass in women as well as the levels of growth hormone and IGF and well preserved physiological menstrual cycle (4). Low energy availability (with or without nutrition disturbance), amenorrhea and osteoporosis, individually or linked as the »Female Athlete Triad« syndrome pose a significant health hazard for sportswomen and physically active women (1). It is a well known fact that sportswomen with irregular menstrual cycle are exposed to the risk of diminished bone mineral density (BMD) (5, 6). Consequentially, osteoporosis may appear, and the BMD can be reduced to the level where stress fractures appear even after the mildest pressure on the bone (7, 8). Measuring BMD via the osteodensimetry method provides just a statical picture of the bone situation. Monitoring of the levels of biochemical markers of bone metabolism enables the understanding of the dynamic changes during the bone remodeling process, and it also has diagnostic value when stress fractures appear (9–11). One of the most sensitive bone formation markers is the serum osteocalcin (OC), an important non-collagen bone matrix protein. The degradation product C of the terminal telopeptide collagen type I, the fragment β-CTx, is a highly specific indicator of bone disintegration (9, 11).

The objectives of the conducted research were 1) to determine the prevalence of menstrual dysfunctions in a sportswomen sample and a control group, as well as 2) to determine the levels of bone metabolism markers in groups of women with menstrual dysfunctions.

Material and Methods

Interviewed women

The women (n=117) were separated into two groups, the experimental (S) (n=84) and the control group (C) (n=34). The experimental group was comprised of three subgroups of sportswomen who practiced different sports: 34 sportswomen for ball games (basketball and handball), 27 for athletics (short and long range runners) and 23 for sport dances. The control group was comprised of female students of the Medical Faculty of similar age who did not practice sports on a regular basis. Parameters of the descriptive statistics of the groups, age, length of sports engagement, weekly physical burden and the body mass index (BMI) are shown in *Table I*. All interviewees of the experimental group, their coaches

Table I Mean values of age, Body Mass Index (BMI), length of sport engagement and weekly physical burden.

Group	n	Age (years)	BMI kg/m^2	Sports engagement duration	Weekly physical burden (h)
Ball games	34	19.64	22.26 [a]	9.5 [b]	12.20 [c]
Dance	23	17.69	19.53	6.52	7.32
Athletics	27	17.41	19.10	5.18	13.98 [c]
Control group [a] ($p<0.05$) compared with dance and athletics [b] ($p<0.05$) compared with dance and athletics [c] ($p<0.01$) compared with dance	34	20.88	21.13	0	0

as well the interviewees in the control group were given written information on the objectives, course, participation and possible unwanted effects of the research. Prior to the commencement of the research, all interviewees gave voluntary consent to participate in the research and were subjected to a general medical examination. One of the criteria for exclusion from the research was the use of a hormone therapy in order to regulate the menstrual cycle, as well as the use of oral contraceptives or drugs that affect bone metabolism.

Menstrual cycle dysfunction determination

To determine the menstrual profile and dysfunction of the menstrual cycle, we used a very detailed questionnaire from which we obtained data on the menstrual cycle (current characteristics of the cycle, menstrual history from the menarche onwards, use of oral contraceptives, etc.). Definitions of menstrual dysfunctions (delayed menarche, oligomenorrhea, primary and secondary amenorrhea) complied with the current recommendations for the field (ACSM Position Stand: The Female Athlete Triad 2007) (1). After that, there was a medical gynecological examination to rule out any other cause of menstrual dysfunctions.

Taking blood and sample analysis

Blood samples were taken by venipuncture of the brachycephalic vein, before meal, in the morning hours, 8–9 am. Immediately after it was taken, the blood was centrifuged, thereby separating the serum, and frozen at –20 °C until the analysis. Prior to the freezing, the samples were checked for hemolysis since erythrocyte proteases dissolve osteocalcin thereby affecting the level of β-CTx (manufacturer's notice).

After the whole sample was collected, the serum was analyzed for specific bone markers. The level of the most stable mid-fragment of osteocalcin (N-MID osteocalcin) as the marker of bone formation was determined, as well as the β-CrossLaps (β-CTx–bone resorption marker) via the electroluminescent immunochemistry method (ECLIA immunoassay) on an Elecsys 1010 automated machine (Roche Diagnostics GmbH, Germany). All the analyses were done at the Physical Medicine and Rehabilitation Institute »Dr Miroslav Zotović«, Banja Luka.

Statistical processing of the results

Depending on the statistical marker, measurement scale, type of distribution, and number and size of samples, the following tests were used: unifactoral variance analysis (ANOVA) Student's t-test, F-test (LSD). The SPSS statistical program for Windows (Release 15.0; Chicago, IL,USA) was used to process the results. The statistical significance was set to $p = 0.05$ for all statistical analyses.

Results

The incidence of all menstrual dysfunctions was higher in the groups of sportswomen in comparison to the control group. Primary amenorrhea was found in 7 (8.33%) and oligomenorrhea in 11 (13.09%) sportswomen. That was a statistically much higher incidence ($p<0.05$) than in the control group where there were no menstrual dysfunctions. Statistically, the highest incidence of menstrual dysfunctions ($p<0.05$) was found in the athletics group, in comparison to the other groups of sportswomen, where primary amenorrhea was detected in 7, and oligomenorrhea in 8 women. The age of the first menstrual cycle showed statistically significantly older menarcheal age in the group of sportswomen than in the control group ($p<0.05$). In the athletics group, menarcheal age was statistically significantly delayed in comparison to the control group and the other groups of examined sportswomen ($p<0.05$). The results regarding the incidence of menstrual dysfunctions are presented in *Table II*.

The intensity of bone metabolism was statistically much higher in the group of sportswomen with menstrual dysfunctions than in the eumenorrheic sportswomen and the control group. The bone metabolism markers values are shown in *Table III*. Statistical significance of the difference was $p<0.01$ at all levels of comparison.

Table II Menstrual cycle dysfunctions among the women examined in the experimental and the control group.

Group	n	Primary amenorrhea	Oligomenorrhea	Age of menarche (years)
Experimental group (S) (Athletes)	84	7*	11*	13.34 *
Control group (C)	34	0	0	12.73
Ball-games (basket/handball)	34	0	2	13.22
Athletics (middle- and long-distance runners)	27	7*	8*	14.27 *
Dance	23	0	1	12.53

* ($p< 0.05$)

Table III Bone metabolism markers values in eumenorrheic sportswomen, sportswomen with menstrual dysfunctions and the control group.

Marker	Group	N	M	SD
β-CrossLaps ng/mL	Eumenorrhea (S) Oligomenorrhea (S) Primary amenorrhea (S) Control (C) *p<0.01	65 11 7 34	0.7618* 0.9788* 1.5101* 0.5421*	0.24713 0.33758 0.50328 0.15818
Osteocalcin ng/mL	Eumenorrhea (S) Oligomenorrhea (S) Primary amenorrhea (S) Control (C) *p<0.01	65 11 7 34	49.5448* 67.6191* 133.7157* 36.3547*	16.17241 27.01311 44.25049 7.26406

Discussion

Premature osteoporosis is a frequent and serious health problem among sportswomen. It is often accompanied with nutrition and menstrual cycle dysfunctions thus constituting a syndrome often called »Female Sports Triad« (12–14). Functional hypothalamic amenorrhea (HPA) is a condition characterized by the absence of menstrual cycle due to suppression of hypothalamic-pituitary-ovarian axis, without the presence of morphological or organic defects (5). Even though the pathophysiology of this condition is not fully explained, there are three conditions related to HPA; stress, loss of body mass and intensive physical activity (5, 15, 16). All three conditions are often present in sportswomen. Professionalism in sport, accompanied with increased intensity, length and frequency of training, causes significant changes in the metabolism and reproductive functions of sportswomen. Estrogen deficiency caused by HPA prevents the generation of peak bone mass in young women and can significantly reduce the positive effects of physical activities on the bone (5, 8, 17). Calcium and protein deficiency due to nutrition disturbance, as well as delayed menarche further enhance premature osteoporosis in sportswomen (18). Consequently, there is a hypothesis that sportswomen with menstrual dysfunctions have an accelerated bone metabolism. Analysis of the frequency of menstrual dysfunctions in the sample covered by the research shows a statistically significantly higher number of interviewees with primary amenorrhea and oligomenorrhea in the group of sportswomen in comparison to the control group. This is particularly so in the group of athletic women – long and mid-range runners. The results are partially in line with numerous studies to date which also point out an increased incidence of menstrual dysfunctions in physical endurance sports (athletics, cycling), weight category sports (judo, wrestling, karate) and esthetic sports (dance, ballet) (15, 19–21). Fewer menstrual dysfunctions in the sport dance subgroup are probably due to lower intensity of the training burden, which also conforms to a lower competition ranking of these interviewees. Previous researches documented the influence of hypoestrogenism on the increase of the level of bone resorption markers, reduction of BMD and the consequential occurrence of osteoporosis in postmenopausal women (22–24). However, very few researches have targeted the dynamics of bone metabolism markers in sportswomen with menstrual dysfunctions or secondary osteoporosis (25–28). Since estrogen suppresses remodeling of the bone and acts antiresorptively, reduction of its level may turn the bone metabolism in the resorption direction, with a prevalence of its markers (4, 28). Values of bone metabolism markers in our study showed a statistically significant difference in the level of bone resorption marker, β-CrossLaps, between the groups of amenorrheic and oligomenorrheic sportswomen in comparison to eumenorrheic women, both sportswomen and those in the control group. Values in the groups of interviewees with menstrual dysfunctions were significantly above the recommended upper values (β-CTx 0.299 ng/mL and OC 15–46 ng/mL) (24). Accelerated resorption was accompanied with accelerated bone formation, which is obvious from the increased osteocalcin level value. However, the values of β-CTx in amenorrheic and oligomenorrheic women were increased to such an extent (β-CTx 1.501 ± 0.50 ng/mL and β-CTx 0.9788 ± 0.33 ng/mL) that they surpassed the values found in women with diagnosed postmenopausal osteoporosis (24). These findings indicate a disturbed balance between the formation and the resorption of bones in the examined sample, negative bone metabolism and possibility of bone mass loss. The β-CrossLaps value in the athletic women subgroup was extremely high, 1.03 ng/mL, which is almost twice the value in the control group, and more than 30% higher than in the eumenorrheic sportswomen. Athletic women are a subgroup of sportswomen with the most frequent menstrual dysfunctions in our research. Similar results were obtained by Herrmann and Herrman (26) who also found a statistically significant value of bone resorption markers in amenorrheic sportswomen

(26). Contrary to our results, Zenker and Swaine (27) with associates, in an earlier study (1998), found a slowed-down bone metabolism in amenorrheic women, but the marker that they measured was the urinary ratio of Deoxsipiridinolin/Creatin (Dpyr/Cr) and osteocalcin. Likewise, Misra (28) listed a slowdown of bone metabolism in amenorrheic sportswomen measuring the C prototype of collagen I (P1CP) and the resorption product N of the terminal telopeptide (NTX), and the condition was explained by a negative energy balance in these sportswomen. Gibson et al. (29) also found a reduced level of osteocalcin when they observed the bone metabolism markers and BMD in 50 British female mid and long range runners. They found that the level of osteocalcin was significantly reduced in the group of amenorrheic women (29). There are data pointing out the influence of surface, that is, that activities with great impact on the bone and increased BMI can reduce potentially dangerous hormone changes in amenorrheic sportswomen (30, 31). However, Misra (28) maintains that this influence is not sufficient to offset the bone loss due to HPA. What limited our study was the inability to measure BMD and consequently confirm the morphological status of the bone. Likewise, even though the research included elite sportswomen (national champions in handball and basketball and 17 members of the national athletics team) we were unable to find a sufficient number of menstrual dysfunctions to put forth more serious conclusions. A combination of nutrition disturbance and reduced intake of calcium and menstrual dysfunctions is the most potent cause of body mass loss in sports. However, not all amenorrheic sportswomen are stricken. Their bone status depends on the type and length of duration of menstrual dysfunctions as well as the factors that exert influence of the bone before their onset (1, 32). Prevention, early recognition of Triad symptoms, vigorous treatment of all symptoms and, of course, comprehensive research of this complex problem will yield the best results in the exhibition of maximally positive effects of physical activity.

It can be concluded that menstrual dysfunctions, delayed menarche, oligomenorrhea and primary amenorrhea are statistically more present in the sportswomen group than in the control group. The greatest incidence of menstrual dysfunctions was noted in the athletics sportswomen group, and it is statistically higher observing delayed menarche, primary amenorrhea and oligomenorrhea in comparison with the other two groups of sportswomen. Menstrual dysfunctions were accompanied with accelerated bone metabolism from the point of view of the increase of bone metabolism markers level. A statistically significantly higher value of the bone resorption maker, β-CrossLaps, was found in the group of amenorrheic and oligomenorrheic sports women in comparison to eumenorrheic sportswomen and the control group. Accelerated resorption was accompanied by increased bone generation (osteocalcin value), but to a lesser degree than the resorption (β-CrossLaps value).

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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