

Biochemical markers of bone turnover in girls with secondary amenorrhoea

J. Krawczyk¹, S. Sajdak², J. Piątek³

¹Department of Clinical Biochemistry,

²Department of Gynaecological Surgery,

³Department of Physiology, Karol Marcinkowski University of Medical Sciences, Poznań (Poland)

Summary

Our aim has been to evaluate the influence of menstrual disturbances of the secondary amenorrhoea type on the metabolism of bone tissue. We pursued this goal by determining selected biochemical markers of bone metabolism. Alkaline phosphatase and osteocalcin were used as indices of bone remodelling. Deoxypyridinoline was used as an index of bone tissue resorption.

Key words: Secondary amenorrhoea; Biochemical markers of bone turnover.

Introduction

The aim of this study was to assess the influence of secondary amenorrhoea on the mineral density of bone tissue in girls after forced weight loss. Our aim was pursued through determination of osteocalcin (OC), total alkaline phosphatase (T-ALP) and deoxypyridinoline (D-PYR). Estradiol (E₂) was determined to confirm proper matching of the study groups. Assessment of bone mineral density was performed in the lumbar spine.

Material and Methods

We studied a group of 27 girls, aged 18 to 26 years, who were hospitalised in the Department of Gynaecological Endocrinology of Karol Marcinkowski University of Medical Sciences Poznań in the years 1997-1998 because of secondary amenorrhoea following forced weight loss. Only the patients who had had no menstruation for at least three months before the launch of the study were enrolled to the study group. The control group comprised 12 girls whose menstruation was regular and whose body weight was adequate for their age.

Information on menstrual cycles, course of amenorrhoea and loss of body weight were obtained from each patient. For each girl height and body weight were measured before the beginning of the study.

Biochemical and hormonal tests

In the control group blood was sampled in the middle of the follicular phase. T-ALP activity was measured by a kinetic method using "Boehringer Mannheim" apparatus. The results were given in international standard units - per litre (U/l). For OC determination the ELISA - OSTEO of "CIS bio international" was used. Measurements were made by the radioimmunochemical method. Results were given in ng/ml. Assessment of D-PYR fraction was performed on the second urine sample in both groups with the "Pyrilinks-D" set used for *in vitro* examination ("Metro Biosystems"). Monoclonal antibodies were used in the set and the measurement was made by an immunoenzymatic method. In the final interpretation of results of D-PYR deter-

minations, the degree of urine concentration was taken into account based on the analysis of creatinine concentration in a given sample. The results were given in nM/mM. E₂ was determined in the serum of both groups by a radioimmunochemical method using the RIA KIT set ("Orion Diagnostica").

Densitometric measurements

Measurement of bone mineral density was performed with Lunar DPX apparatus. The mineral density of bones (BMD) was measured in the AP projection of a single vertebrae and of combined areas of the L₁ - L₄ segment of the spine. The statistical error did not exceed 1%.

Statistical analysis

The analysed parameters are given as the arithmetic mean (x) and standard deviation (SD). The Shapiro-Wilk test was used to check compatibility of the studied parameters with the normal distribution. Calculations were made at the Department of Medical Statistics in Poznań using the STATISTICA v 5.0 software.

Results

The following parameters were analysed: age, body mass, weight loss, body mass index (BMI), and duration of amenorrhoea. Biochemical, hormonal and densitometric tests were also carried out. Osteocalcin concentration in the serum of the study group was considerably higher ($p < 0.05$) than in the control group. The mean OC concentration in the study group was 20.24 ± 5.30 ng/ml, whereas in the control group it was 16.60 ± 2.10 ng/ml (Figure 1). In the group of hospitalised girls no correlation was observed between osteocalcin concentration and the duration of amenorrhoea and the BMD values in the L₂ - L₄ part of the spine. Total serum alkaline phosphatase activity in the study group with secondary amenorrhoea after forced weight loss (average value: 67.70 ± 20.70 U/l) did not differ statistically from the value in the group with regular menstruation (65.00 ± 13.77 U/l). T-ALP values are presented in Figure 2. Absence of significant

Revised manuscript accepted for publication April 8, 2002

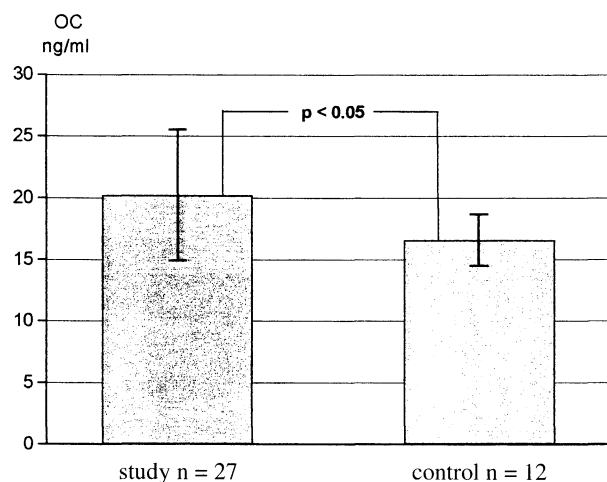


Figure 1. — Comparison of the values of osteocalcin concentrations in the study and control groups.

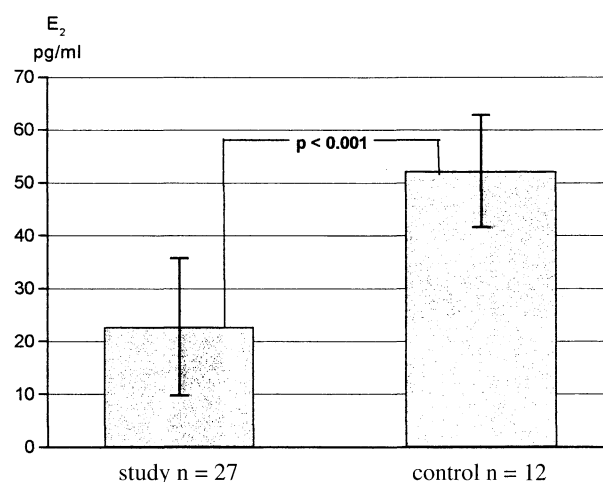


Figure 4. — Comparison of estradiol concentration value in the study and control group girls.

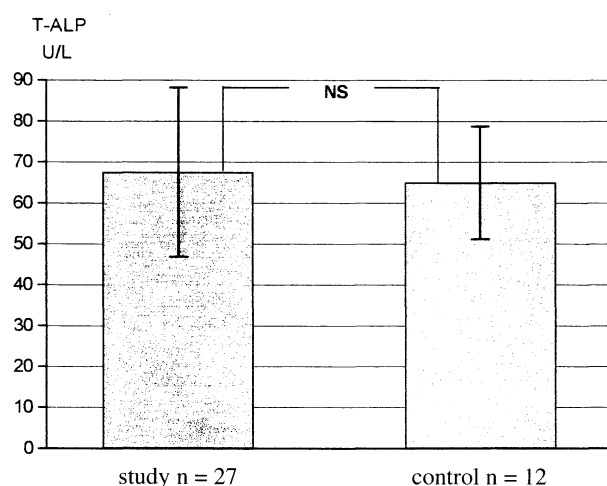


Figure 2. — Comparison of total alkalic phosphatase activity in the hospitalised girls and in the controls.

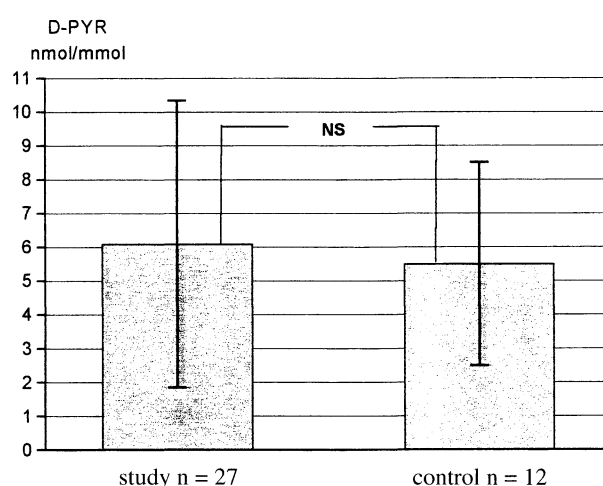


Figure 5. — Comparison of the BMD values in girls in the hospitalised and control groups.

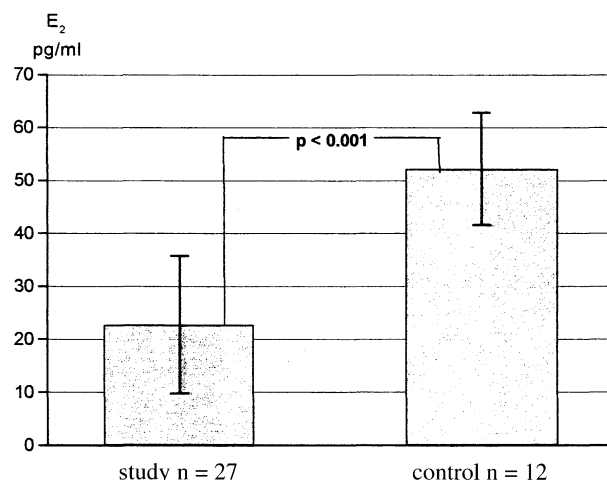


Figure 3. — Comparison of deoxypyridinoline concentrations in the hospitalised girls and the controls.

differences in T-ALP concentrations between the study and the control group confirms the view held by other authors that T-ALP as a marker of the remodelling of bone tissue is not very sensitive and nonspecific for the tissues.

Average deoxypyridinoline concentration in the study group was 6.10 ± 4.24 nM/mM compared to 5.51 ± 3.01 nM/mM in the control group. No statistically significant differences in concentrations were shown between the hospitalised girls and the control group (Figure 3). 17- β estradiol concentration in the hospitalised group was on average 22.78 ± 13.00 pg/ml and was considerably lower ($p < 0.001$) than in the control group (52.25 ± 10.61 pg/ml). The results are presented in Figure 4. It can be seen from the analysis of the measurements that the average bone density in the L₁ - L₄ segment of the spine in the study group was 1.034 ± 0.175 g/cm², whereas in the control group it equalled 1.234 ± 0.145 g/cm² with the difference being significant ($p < 0.001$).

Assuming that the average value of BMD determined in the control group is 100% and comparing it with the average value of BMD determined for the hospitalised group it was found out that in the group with the secondary amenorrhoea following forced weight loss there was a considerable degree of BMD loss (the difference of 16%).

Discussion

Bone turnover is maintained by the temporary anatomical structure composed of osteoblasts and osteoclasts named a basic multicellular unit (BMU) by Frost [12, 17, 21]. Total bone metabolism is a result of the influence of all the processes of remodelling which occur at a given moment within the whole skeleton and depends on the number of multicellular units of remodelling [20].

Bone resorption markers are assayed in the urine and in the serum, and their diagnostic importance is increasing because so far no reliable methods to determine the rate of bone resorption through bone biopsy have been developed [17]. The latest generation of bone tissue resorption assays are the pyridine binds of type I collagen and type I collagen cross-linked N-telopeptides [8]. It was shown that the results of these measurements correlate with the results of dynamic studies of bone histomorphometry and calcium kinetics [2]. At present it is assumed that there are three indications for determination of biochemical markers of bone metabolism [17, 21]:

Predicting bone mass loss

A direct link was revealed between accelerated metabolism of bone tissue and the increased rate of bone loss [11, 12, 14, 21];

Predicting treatment-induced changes in bone mass

Bone turnover markers seem to be useful in monitoring of results of treatment whose aim is to prevent bone resorption in patients with osteoporosis [14, 16, 20, 21];

Predicting bone fractures

Garnero, Melton and Kleerekoper refer to prospective studies which show that the rate of metabolic turnover is also a prognostic index for osteoporotic fractures and is independent of the initial bone mass [11, 12, 16, 18].

Other reports point out the usefulness of determination of indices of bone turnover in predicting the individual rate of bone mass loss and, correlating with it, the individual risk of osteoporosis [1, 5, 8, 14, 19]. The majority of researchers who have assessed the prognostic value of biochemical indices of bone turnover in individual patients also agree that a single measurement is not sensitive enough for osteoporosis screening [5, 6, 15]. Clinically more useful information is yielded by the combination of the BMD measurement and bone turnover markers as well as assessment of bone metabolism in the selected population groups [1, 3, 10]. Taking into consideration that biochemical indices of metabolism cannot

be the basis for diagnosis of osteoporosis, we can still say that their usefulness has been proven and that these are sensitive and specific tests for assessing patients' reactions to antiresorptive treatment [10, 11, 13, 15]. Bone tissue is highly estrogen-dependent. Minimal E_2 concentration which stimulates bone tissue ranges from 50 to 60 pg/ml and each form of hypoestrogenism significantly increases the activity of the multicellular unit of bone remodelling, thus accelerating BMD loss [4, 17, 22]. This pathomechanism is of key importance in the pathophysiology of osteopenia and osteoporosis observed in girls with menstrual disturbances and concomitant hypoestrogenism [2, 8, 17, 22].

Conclusions

1) In girls with secondary amenorrhoea induced by forced weight loss the bone mass loss was found to co-occur with the increase in the concentration osteosynthesis indices.

2) Secondary amenorrhoea induced by forced weight loss does not lead to an increase in the concentration of indices of osteolysis.

3) Hypoestrogenism observed in girls with secondary amenorrhoea induced by forced weight loss is correlated with a considerable bone mass loss and is an important risk factor for osteoporosis.

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Address reprint requests to:
JACEK PIĄTEK M. D. Ph. D.
Dept. of Physiology
Karol Marcinkowski University
of Medical Sciences
Ul. Świecickiego, 6
60-781 Poznań, Poland