DYNAMIC PATTERN OF IGF-I AND CHOSEN BIOCHEMICAL MARKERS OF BONE METABOLISM IN A RAT MODEL OF POSTMENOPAUSAL OSTEOPOROSIS

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Objective. To assess the relationship between the dynamic pattern of IGF-I levels and chosen biochemical markers of bone metabolism in ovariectomized rats – a model of postmenopausal osteoporosis.

Methods. Six-month-old rats were randomized to sham operation (control group) or ovariectomy. Serum levels of insulin-like growth factor (IGF-I) and chosen biochemical markers of bone metabolism (alkaline phosphatase – ALP, carboxyterminal propeptide of type I procollagen – PICP, cross-linked carboxyterminal telopeptide of type I collagen – ICTP in serum as well as urinary excretion of hydroxyproline – HYP and total calcium – Ca) were measured before (group 0) and 1, 2, 3, 4, 6, 8, 12, 16, 20, 24 and 28 weeks after the operation.

Results. In a model of experimental osteoporosis induced by ovariectomy in female rats a distinct tendency to decrease the IGF-I concentrations was shown. Differences were significant in relation to the control group in a period from 2 to 28 weeks after operation. Ovariectomy stimulated the values of studied markers of bone metabolism; it was more intensified in regard to resorption markers. Significant ICTP and HYP concentrations' changes, in relation to the control group, were shown in the some period and total calcium – from 2 to 16 weeks after ovariectomy. However, the values of studied markers of bone formation were generally changing to a slight degree. Significant differences of ALP activity, in relation to the control group, were observed only at 8 and 20 weeks, while those of PICP concentrations were found at in 4, 8 and 12 weeks after the operation. The alterations in the levels of IGF-I correlated significantly and negatively with the changes in ALP activity as well as in PICP, ICTP, HYP and Ca concentrations both in ovariectomized and control rats. This relation was more xpressed in the ovariectomized group.

Conclusions. Our findings suggest that secondary changes in IGF-I concentration, due to the deficiency of sex hormones, results in altered bone metabolism in ovariectomized rats.

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Key words: IGF-I – Bone metabolism – Model of postmenopausal osteoporosis – Female rats

Although the causes of postmenopausal osteoporosis are not definitely elucidated, the time relation to menopause suggests that the deficiency of estrogens apparently plays an essential role. It is still not fully clarified in what way it leads to excessive bone tissue resorption (Badurski 1994; Galus 1995). It is known that estrogens can influence bone tissue metabolism indirectly, by inducing changes in concentration of systemic and local factors such as: calcitonin, interleukin-1 and 6 (IL-1 and IL-6), transforming growth factor-β (TGF-β), prostaglandin-E_2 or insulin-like growth factors (IGFs), especially IGF-I (Paciifici et al. 1987; Ernst et al. 1989; Gray et al. 1989; Stock et al. 1989; Ernst and Rodan 1991;

Kajdaniuk et al. 1999). It is also claimed that osteoblasts and osteoclasts have receptors to estrogens which suggests the possibility of its direct influence on bone tissue (Komm et al. 1988; Kaye et al. 1990; Badurski 1994; Galus 1995; Kajdaniuk et al. 1999). Estrogens stimulate the autocrine secretion of TGF-β and IGF-I by osteoblasts which further stimulates the maturation and growth of osteoblasts as well as collagen and alkaline phosphatase secretion (Badurski 1994; Galus 1995; Marek et al. 1996; Kajdaniuk et al. 1999).

It is known that IGF-I secretion is controlled by several different regulatory factors, such as: hormones, growth and immunological factors (Canalis et al. 1987, 1988, 1989; Centrella et al. 1988, 1993; Hall and Tally 1989; Linkhart and Mohan 1989; Schmidt et al. 1989; Kream et al. 1990; McCarthy and Centrella 1990; Aaronson 1991; Linkhart and Keffer 1991; Shimasaki and Gao 1991; Mohan and Baylink 1993; Raisz et al. 1993; Gohel et al. 1995; Marek et al. 1996; Rosen 1999, 2000). The final adult level of IGF-I represents the sum of the inert circulating depot, newly derived IGF-I synthesized from various tissues (including liver, heart, kidney, bone, and others), and the departure from the circulation of IGF-I through various pathways including receptor internalization and proteolysis of several insulin-like growth factors binding globulins (IGFBPs) (Rosen 1999, 2000). Although there is a dose-response relationship between IGF-I and bone mass (Bennett et al. 1984; Romagnoli et al. 1993; Wüster et al. 1993; Warenik-Szymankiewicz 1997), or risk fractures, it is not clear that it is causal. Yet, it is uncertain whether low levels of circulating IGF-I actually cause osteoporosis. Moreover, it has not been proved that serum levels of this peptide always reflect various tissue concentrations (Rosen 1999, 2000).

The aim of the undertaken studies was: 1. to show if, in what degree and in what time after ovariectomy in female rats (experimental model of postmenopausal osteoporosis) disturbances in IGF-I secretion can occur; 2. to establish if possible changes in IGF-I concentrations can have a significance in generating expected disturbances of bone metabolism (as evaluated on the base of assays of chosen biochemical markers of bone tissue formation such as alkaline phosphatase - ALP and carboxyterminal propeptide of type I procollagen - PICP in serum) and resorption (cross-linked carboxytermnal telopeptide of type I collagen - ICTP in serum as well as hydroxyproline - HYP and total calcium – Ca in urine).

Materials and Methods

Studies were performed in 138 adult female Wistar rats with an initial body mass of 145 ± 9 g. During the experiment the animals were housed under conditions of uniform temperature (20-22 °C), air humidity (80-85 %) and light (LD 12:12; light from 7.00 to 19.00 h). The animals were fed at the onset of the dark phase using a standard diet suitable for conducting bone metabolism research in experimental animals (Altromin Standard Diäten, Austria) and were given filtered drinking water *ad libitum*.

After two weeks of adjustment, vaginal smears were taken daily between 8 and 8.30 am. After establishing the estrus cycle, 6 animals were left intact (group 0), 66 underwent ovariectomy (Ovx) in the estrus phase and the remaining ones underwent a sham operation (C - control group). Two weeks after surgery, both ovariectomized and control rats were divided into 11 equal subgroups of 6 animals in each. At the appropriate time, i.e. prior to surgery (group 0) and 1, 2, 3, 4, 6, 8, 12, 16, 20, 24 and 28 weeks after operation, the animals were marked and placed separately in metabolic cages for 3 hours (from 6.30 until 9.30 h) in order to collect urine aliquots for hydroxyproline (HYP) and total calcium (Ca) determinations. The rats were decapitated at 8.00 h on the following day. The blood was collected into test tubes with granulated mass (Sarstedt) and centrifuged immediately. The obtained sera were stored frozen at -75 °C until determination of 17β-estradiol (E2) and insulin-like growth factor (IGF-I) levels as well as alkaline phosphatase (ALP) activity and carboxyterminal propeptide of type I procollagen (PICP) and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) concentrations.

Serum concentrations of hormones, PICP and ICTP in female rats studied were measured with commercially available RIA kits: E2 (Immunotech, France), IGF-1 (DSL, USA), PICP and ICTP (Farmos, Finland). Serum ALP activity (temperature of 37 °C) and urinary excretion of Ca were determined using Alpha-Diagnostic kits (Poland). Urinary excretion of HYP was determined according to Drozdz

et al. (1976). The sensitivity of assays was as follows: E2 – 5.0 pmol/l, IGF-I – 2.73 nmol/l, PICP – 1.2 μg/l, ICTP – 0.34 μg/l, HYP 6.6 μmol/l. The linearity for ALP and Ca methods were up to: 1000 U/l and 16 mg/dl, respectively. The respective intraassay and interassay coefficients of variations were: E2 – 5.1 and 7.9 %, IGF-I – 3.7 and 6.1 %, PICP – 3.1 and 5.8 %, ICTP – 4.5 and 6 %, HYP – 5.5 and 7.2 %, ALP – 7.8 and 8.5 %, Ca – 4.7 and 6.8 %.

Statistical evaluation. The results were eavluated using variance analysis for parametric tests when distribution of the variable was normal. When the variable distribution deviated from normal, the variance analysis was conducted according to the Kruskal-Wallis method for nonparametric tests. The interrelation between IGF-I levels and the values of chosen biochemical markers of bone metabolism was assessed using the Pearson correlation test (when variable distribution was normal) or the Spearman correlation test (when variable distribution departed from normal).

Results

In all rats with removed ovaries (experimental model of postmenopausal osteoporosis) and, resulting from this operation reduced concentrations of E2, it has been found that there are dynamic, significant changes in values of IGF-I and chosen biochemical markers of bone metabolism. These alterations were dependent on the time after the operation (Table 1, Figure 1).

In ovariectomized rats a suppression of IGF-I concentrations was shown. It was revealed in the second week after the operation and maintained till the 28th week after the operation.

Ovariectomy influenced stimulatively on values of studied markers of bone metabolism; it was more intensified in regard to resorption markers, especially ICTP and HYP. Significant ICTP and HYP concentrations' changes, in relation to the control group, were shown in a period from 2 to 28 weeks and Ca - from 2 to 16 weeks after ovariectomy. However, the values of studied markers of bone formation were changing only to a slight degree. Significant differences of ALP activity, in relation to the control group, were observed only in the 8th and 20th week, and of PICP concentrations – 4, 8 and 12 weeks after the operation.

The analysis of correlation has shown that the alterations in the concentrations of IGF-I correlated, significantly and negatively with changes in ALP activity as well as PICP, ICTP, HYP and Ca concentrations both in control (except HYP) and ovariectomized rats. This relation was more intensified regarding the ovariectomized group (Table 2).

Discussion

Although osteoporosis induced by ovariectomy in female rats has been widely used as a suitable model for postmenopausal osteoporosis in humans, the findings of different authors are not always consistent (Badurski 1994; Galus 1995). This could be mainly due to differences in the age of the studied animals, the time after ovariectomy, and measured markers (Gurkan et al. 1986; Yamazaki and Yamaguchi 1989; Dick et al. 1996).

In a model of experimental osteoporosis induced by ovariectomy in female rats a distinct tendency to increase the level of studied biochemical markers of bone metabolism was shown; it was more pronounced in regard to resorption markers, especially ICTP and HYP. However, the values of studied markers of bone formation were generally changing in a slight degree. Our results indicate that ovariectomy and decreased estrogen can have essential significance in inducing balance disturbances between the processess of bone resorption and bone formation leading in effect to loss of bone mass. Although there is evidence that estrogen deficiency is an important contributory factor, the pathogenesis of postmenopausal osteoporosis is multifactorial and presently poorly understood (Badurski 1994; Galus 1995). IGF-I seems to play an important role in the development of age-related bone loss (Bennett et al. 1984; Romagnoli et al. 1993; Wuester et al. 1993; Warenik-Szymankiewicz 1997). Recent reports have indicated that reduced plasma levels of IGF-I are associated with osteoporosis in both males and females (Rosen 1999, 2000; Calo et al. 2000). Several authors suggest that IGF-I is a useful predictor of the presence of postmenopausal osteoporosis (Celiker and Arslan 2000; Zofkova et al. 2001).

IGF-I had been shown to be an important stimulator of bone formation and osteoblast activities *in vit-*

Table 1

Dynamic pattern of serum 17 β estradiol (E2) and insulin-like growth factor-I (IGF-I) concentrations, alkaline phosphatase (ALP) activity, carboxyterminal propeptide of type I procollagen (PICP) and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) concentrations as well as urinary excretion of hydroxyproline (HYP) and total calcium (Ca) in ovariectomized (Ovx), sham operated (C – control group) and intact rats (0)

Variables	Groups	Time (weeks)											
		0	1	2	3	4	6	8	12	16	20	24	28
E2 pmol/l	C	93.89 ± 10.08	97.85 ± 10.19	102.96 ± 9.20	99.64 ± 8.14	102.60 ± 10.15	94.21 ± 11.10	102.58 ± 9.18	99.91 ± 12.12	102.62 ± 14.15	94.11 ± 9.07	96.85 ± 8.06	89.23 ± 11.21
	Ovx		**18.45* ± 2.96**	**13.18* ± 2.09**	**9.01* ± 0.95**	**14.56* ± 1.16**	**18.38* ± 2.20**	**22.58* ± 2.17**	**14.46* ± 3.11**	**9.01* ± 1.19**	**11.32* ± 1.17**	**12.00* ± 0.10**	**16.05* ± 2.04**
IGF-I nmol/l	C	258.00 ± 35.97	254.88 ± 37.61	256.33 ± 29.86	249.79 ± 37.84	255.20 ± 3.65	241.80 ± 33.06	245.01 ± 3.65	245.63 ± 36.81	239.23 ± 28.28	240.48 ± 21.63	235.80 ± 20.67	263.66 ± 29.84
	Ovx		231.55 ± 34.13	**208.75* ± 31.25**	**175.18* ± 29.80**	**169.47* ± 42.52**	**170.67* ± 34.26**	**166.22* ± 28.74**	**199.57* ± 55.45**	**189.82* ± 31.02**	**209.15* ± 20.21**	**205.13* ± 22.98**	**200.09* ± 26.57**
ALP U/l	C	199.01 ± 20.17	188.35 ± 19.30	184.42 ± 27.12	182.63 ± 40.21	178.67 ± 21.18	187.26 ± 21.10	167.95 ± 29.86	178.92 ± 28.97	166.62 ± 28.53	150.19 ± 17.92	152.64 ± 39.73	150.95 ± 30.04
	Ovx		175.32 ± 29.23	213.00 ± 20.25	198.72 ± 29.48	217.24 ± 29.74	200.84 ± 29.89	**210.38* ± 13.09**	187.26 ± 2.12	184.61 ± 39.17	**182.82* ± 10.11**	186.49 ± 38.62	180.39 ± 39.24
PICP µg/l	C	9.75 ± 0.79	9.54 ± 0.76	8.12 ± 1.06	10.37 ± 1.22	9.76 ± 1.26	9.63 ± 1.74	9.82 ± 0.54	9.41 ± 0.46	10.35 ± 0.45	9.87 ± 0.75	9.41 ± 0.66	9.91 ± 0.98
	Ovx		9.62 ± 1.26	9.09 ± 0.31	10.94 ± 1.02	**11.36* ± 0.83**	10.73 ± 1.11	**10.95* ± 0.84**	**10.32* ± 0.75**	10.96 ± 0.64	10.82 ± 0.77	10.27 ± 0.93	10.82 ± 0.67
ICTP µg/l	C	14.73 ± 0.69	14.53 ± 1.65	13.15 ± 1.16	15.35 ± 1.14	14.54 ± 1.78	16.47 ± 1.55	17.23 ± 1.46	14.46 ± 1.10	15.87 ± 1.15	14.96 ± 0.90	14.02 ± 0.87	14.91 ± 2.02
	Ovx		16.29 ± 1.16	**21.91* ± 1.64**	**20.05* ± 1.06**	**21.14* ± 2.87**	**21.33* ± 1.14**	**21.27* ± 1.12**	**20.33* ± 1.02**	**20.72* ± 1.27**	**19.96* ± 0.89**	**19.43* ± 0.90**	**19.17* ± 1.41**
HYP µmol/l	C	16.00 ± 1.91	15.34 ± 1.94	16.32 ± 1.54	15.34 ± 1.87	14.78 ± 1.47	15.80 ± 1.48	14.42 ± 1.97	14.73 ± 1.73	16.92 ± 1.60	17.35 ± 1.32	17.43 ± 1.40	17.14 ± 1.42
	Ovx		18.34 ± 2.83	**19.23* ± 1.72**	**20.58* ± 1.97**	**23.02* ± 1.70**	**23.17* ± 1.55**	**24.23* ± 1.83**	**22.14* ± 1.65**	**22.26* ± 1.80**	**21.54* ± 1.52**	**21.41* ± 1.76**	**20.85* ± 1.73**
Ca mmol/l	C	2.72 ± 0.23	2.70 ± 0.51	2.53 ± 0.45	2.71 ± 0.54	2.64 ± 0.30	2.86 ± 0.26	3.38 ± 0.19	3.34 ± 0.25	3.11 ± 0.28	3.37 ± 0.47	2.92 ± 0.84	3.24 ± 0.32
	Ovx		3.37 ± 0.47	**3.35* ± 0.39**	**3.94* ± 0.40**	**3.99* ± 0.38**	**4.30* ± 0.29**	**4.25* ± 0.31**	**4.28* ± 0.28**	**4.12* ± 0.20**	3.73 ± 0.45	3.82 ± 0.57	3.76 ± 0.48

$p \leq 0.05$ vs control group

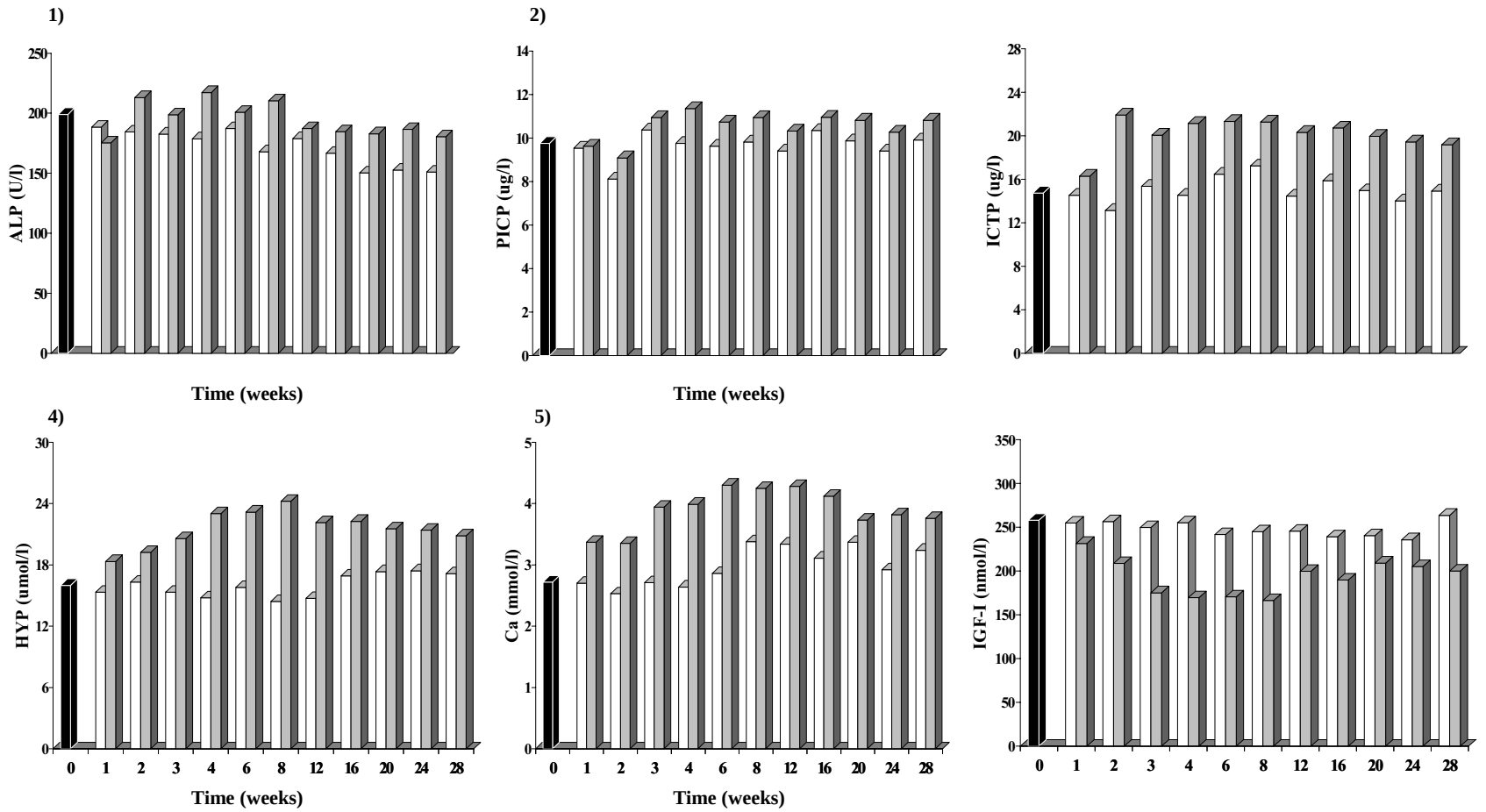

Fig. 1 Mean serum alkaline phosphatase (1 - ALP) activity, carboxyterminal propeptide of type I procollagen (2 - PICP), cross-linked carboxyterminal telopeptide of type I collagen (3 - ICTP) concentrations, urinary excretion of hydroxyproline (4 - HYP) and total calcium (5 - Ca) as well as serum insulin-like growth factor-I (6 - IGF-I) levels in ovariectomized (Ovx) or underwent sham operation female rats (C - control group), killed before (0 group) and 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28 weeks after the operation

Table 2. Correlation between dynamic pattern of insulin-like growth factor-I (IGF-I) and 17β-estradiol (E2) as well as chosen biochemical markers of bone metabolism in sham operated (C) and ovariectomized (Ovx) rats

Independent variable	Dependent variables	C	Ovx
IGF-I (nmol/l)	E2 (pmol/l)	**0.432***	**0.597***
	ALP (U/l)	**– 0.354***	**– 0.674***
	PICP (μg/l)	**– 0.340***	**– 0.706***
	ICTP (μg/l)	**– 0.336***	**– 0.684***
	HYP (μmol/l)	– 0.197	**– 0.807***
	Ca (mmol/l)	**– 0.384***	**– 0.762***

* $p \leq 0.05$ significant values of correlation coefficients

ro and *in vivo* (Canalis et al. 1988, Centrella et al. 1988, 1993; Pascual et al. 1995, Kajdaniuk et al. 1999, Vande et al. 1989, Hock et al. 1988, Goldstein et al. 1989, Kveiborg et al. 2000). It is known that the aging of osteoblasts is associated with impaired production of the stimulatory components of the IGF-system, which may be a mechanism contributing to age-related decline in the osteoblast function (Kveiborg et al. 2000). IGF-I also stimulates bone tissue resorption *in vitro*, having an influence on mature osteoclasts and conditions forming new osteoclasts from precursor cells (Canalis et al. 1988; Centrella et al. 1988, 1993). Ibbotson et al. (1992) studies indicate that IGF-I treatment only slightly reduced bone mineral content in ovariectomized rats and significantly stimulated resorptive activity. IGF-I had no significant effect on bone formation but when combined with PTH, IGF-I blunted the response to PTH on the periosteal and endocortical surface (Ibbotson et al. 1992). IGF-I and/or IGFBP-5 seemed to be involved in the estrogen-induced modulation of PTH action on osteoblast proliferation and function (Nasu et al. 2000). Similarly as the administration of E2, also that of rhGH and rhIGF-I to aged ovariectomized rats prevents further loss of bone mass and strength at sites containing trabecular bone. In addition, rhGH increases cortical bone mass above pretreatment values (Verhaeghe et al. 1996).

In our studies in female rats, which included the period of 28 weeks after ovariectomy, the changes in the values of chosen markers of bone metabolism were accompanied by a distinct suppression of IGF-I concentrations, the differences being significant in relation to the control group in a period from 2 to 28 weeks after the operation. The alterations in the levels of IGF-I in ovariectomized rats correlated significantly and negatively with changes in ALP activity as well as with PICP, ICTP, HYP and Ca concentrations; this relation being more intensified in regard to resorption markers. Our findings in female rats seem to corroborate the concept of secondary changes in IGF-I concentrations co-participating in the development of bone mass changes characteristic for postmenopausal osteoporosis. This conception is also confirmed by the results of clinical studies which show that there is a significant correlation between IGF-I concentration in serum and the decrease of bone tissue density in postmenopausal women (Bennett et al. 1984; Romagnoli et al. 1993; Wuester et al. 1993; Warenik-Szymankiewicz 1997). The deficiency of estrogens presumably results in a decrease of IGF-I secretion, which can lead to the advantage of resorption process over bone tissue formation. A decline in IGF-I during aging has been proposed by most investigators as a causal factor in the development of postmenopausal osteoporosis (Bennett et al. 1984; Pun et al. 1990; Ljunghall et al. 1992; Ramagnoli et al. 1993; Wuester et al. 1993, Warenik-Szymankiewicz 1997; Celiker and Arslan 2000; Wuester 2000). However, in some postmenopausal women decreased serum concentrations of IGF-I were strongly associated with an increased risk of osteoporotic fractures independently of bone-mineral density (Gamero et al. 2000; Seck et al. 2001). Other authors have shown no difference in the levels of components of the IGF-system in patients with osteoporosis (Rosén et al. 1992; Kassem et al. 1994; Gamero et al. 2000).

On the basis of available data it is suggested that the mechanism of IGF-I action on bone tissue *in vivo* is complex. Not only independent effects of IGF-I action can play a role here, but also the interaction

with endogenous factors. It is known that apart from GH also other hormones such as sex steroids, cortisol, insulin, gonadotropins, TSH, PTH and melatonin can influence IGF-I secretion (Hall and Tally 1989, Marek et al. 1996). Also some polypeptide growth factors may play a part in the regulation of GH secretion, so indirectly also IGF-I (Aaronson 1991; Kajdaniuk and Marek 1999). To the most important factors stimulating IGF-I formation in bones PTH belongs (Canalis et al. 1989; Linkhart and Mohan 1989; Linkhart and Keffer 1991), and from local factors – TGF-β (Linkhart and Keffer 1991) and prostaglandin-E2 (Raisz et al. 1993). Beneficial action of β2-microglobulin on the increase of binding IGF-I with osteoblasts is also underlined (Cannalis et al. 1987). Glucocorticosteroids inhibit IGF-I synthesis in bones, which explains its unbeneficial influence on bone mineral density (Schmidt et al. 1989; Kream et al. 1990; McCarthy and Centrella 1990; Gohel et al. 1995). Also 1.25(OH)2 D3 inhibit IGF-I synthesis (Linkhart and Keffer 1991). Binding proteins - IGFBPs (except IGFBP-1) which regulate the influence of IGF-I on osteoblasts are also produced in bone tissue (Canalis et al. 1988; Centrella et al. 1988, 1993; Schmidt et al. 1989; Shimasaki and Gao 1991). Most studies indicate that high IGFBPs concentration act suppressively, and degradation – stimulatingly on IGF-I activity (Mohan and Baylink 1993). The complex interrelationship between the IGF regulatory system and estrogens/androgens in the postmenopausal period may provide important clues as to the pathophysiology of several age and sex hormone related degenerative disorders (Rosen et al. 1998).

Finally, in a model of postmenopausal osteoporosis induced by ovariectomy in female rats a distinct suppression of IGF-I concentrations was shown. Changes in IGF-I concentrations correlated significantly with changes in the values of chosen indices of bone formation and resorption, which can indicate an essential role of this protein in generating changes as far as bone tissue metabolism after menopause is concerned.

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